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CARBON AND NITROGEN DYNAMICS IN FOREST SOILS

DINAMICHE DEL CARBONIO E DELL'AZOTO IN SUOLI FORESTALI

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“The purpose of a model is not to fit the data
but to sharp the questions”.
However
“Experimental data without models are like chaos,
models without experimental data are like fairy
tales”M.R.

Abstract

Soil represents a compartment of utter importance within the context of carbon (C) cycle since constitutes the major C reservoir at global scale, in particular in forest ecosystems, and regulates the processes of the litter decomposition and immobilization of the organic matter controlling the mechanisms of carbon dioxide (CO_2) exchange between the soil interface and the atmosphere. Soil is hence a fascinating compartment on one hand and complex on the other hand with regards to the processes of biochemical nature, their measurements, the several feedbacks and their modellization. The process of litter decomposition is the first step toward the C sequestration within the soil and it is mediated mainly by the activity of microorganisms; the interaction and stabilization within mineral matrix follows. Markedly the biotic component constitutes the most sensitive item to external disturbances of both climatic rather than anthropogenic nature. Presently a lot of attention has moved toward the role of nitrogen (N) and the biochemical effects on the soil decomposition activity and C stock, but the experimental evidence is still not coupled to a general scheme able to explain consistently all the results reported in literature. The present work focuses hence on the dynamics of C and N in to the soil, in particular the effects of a surplus of mineral N on C cycle through the role of microbial community. In a first phase of the study a conceptual model have been proposed after an initial work of review; a more detailed investigation followed by medium of different approaches, including an incubation experiment of a N-fertilized soil and the development of two models. The general introduction has underlined how soil effectively constitutes a crucial issue concerning its contribute to C cycle within forest ecosystems, the response of which to external disturbance such as N deposition or N fertilization is markedly mediated by the significant heterogeneity and hence by the spatial and the temporal scales of the processes. However, a conceptual scheme has been proposed in order to organize all the main findings in literature in a general scheme able to include the observed microbial response to mineral N addition, and more generally to the availability of C and N of different quality. In this conceptual model microorganisms have been considered as a single individual that behaves in order to achieve an optimal and efficient condition, depending on the energetic status of resources regardless the real mechanisms affecting any changes of C and N quality.

A part of the work interested the realization of an incubation experiment of a Mediterranean forest (Quercus cerris) soil subject to N addition. The type of site and the experimental set up with the slow addition of mineral N constituted a novelty within the literature with regards to N fertilization studies in laboratory. In fact this study allowed firstly filling the gap concerning the current knowledge that interested mainly investigations on the forests of North Europe and North America. Although the experiment was conducted in laboratory the soil showed in the first period a response

similar to the response observed in a site covered by an oak-type vegetation in north America, supporting the hypothesis that on old organic matter originated by low-quality litter the recalcitrance may control microbial response to N addition independently on direct control of climatic factors. Indeed these factors regulate mainly the vegetational composition, the litter quality and the rate at which litter is initially degraded. Moreover it has been shown as the process depended on the microbial activity pattern throughout incubation time that affected in a non-linear way the results at the end of incubation. The results suggested how mineral N may be used as key-variable driving the microbial activity on the long temporal scale, overcoming the need to go into deep of specific mechanisms of biochemical nature (i.e. acidification process).

A litter decomposition and organic matter accumulation model was thus developed, starting from a soil C basic scheme. The N cycle was modified in order to take into account the all interactions between the two cycles, in particular with regards to N in both organic and mineral form. The goal of the work was to propose a simple formulation that expresses the N-control on long temporal scale and that can be included in the current dynamic soil model. The merit of the work was to the modelling of a process presently not yet completely included in dynamic models. Indeed the effort was to find a trade off between the complexity of the biochemical processes and the modelling approach than could maintain the most judicious mathematical simplification in order to be manageable and easy to be interfaced with the models currently present in literature. The proposed formulation modifies the decomposition activity as a function of microbial N demand and mineral N availability in to the soil. The model, characterized by a paucity of parameters to be optimized was able to reproduce the experimental evidences reported in literature and underlined the role of soil microorganisms and of the mineral N on the decomposition activity of recalcitrant matter. However it was underlined how the model should need an optimization “ad hoc” in order to be extended to the whole soil profile. Regardless the optimization procedure the proposed model offers an interesting formulation that can be included in the current plant-soil dynamic models.

Finally the last phase of the work was moved toward the modification of a soil model in order to consider the microbial community behaviour on mixed substrates, following the input match law and the achievement of a microbial goal, i.e. maximimation of growth. Population behaviour has been investigated with regards to animal versus resources acquisition at high scales, but it has been rarely applied to the dynamic of soil C-cycle. The latter model, althoght still a theoretical exercise, returned the best performances and adds thus a new dimension to the area of study of soil decomposition models at the plot scale.

Sommario

Il suolo rappresenta un comparto importantissimo all'interno del bilancio del carbonio (C), costituendo la maggior riserva di C a livello globale, in particolare negli ecosistemi forestali, regolando i processi di decomposizione della lettiera e di immobilizzazione della sostanza organica, controllando i processi di scambio dell'anidride carbonica (CO_2) con l'atmosfera. Il suolo è quindi un comparto sicuramente affascinante ed allo stesso tempo complesso, sia per quanto riguarda la parte relativa ai processi biochimici che avvengono al suo interno, la loro misurazione, i vari feedbacks, che per quanto riguarda la sua modellizzazione. La decomposizione della lettiera costituisce il primo step verso il sequestro di C nel suolo ed è mediato principalmente dall'attività dei microorganismi, a cui segue l'interazione e la stabilizzazione con la matrice minerale. In particolare la componente biotica costituisce il fattore più sensibile a disturbi esterni, di qualunque natura, legati sia a fattori climatici piuttosto che antropici. Allo stato attuale molta attenzione è stata rivolta al ruolo dell'azoto (N) ed agli effetti biochimici sull'attività di decomposizione ed al sequestro di C, ma l'evidenza sperimentale non è stata ancora accompagnata da una spiegazione generale che potesse mettere d'accordo tutti i risultati in modo consistente. Il presente lavoro è stato focalizzato quindi sulle dinamiche di C ed N all'interno del suolo in particolare gli effetti del surplus di N minerale, passando attraverso il ruolo della comunità microbica. In una prima fase di lavoro è stato sviluppato un modello concettuale dopo un lavoro approfondito di review; è seguita quindi una parte di studio più dettagliata con un approccio metodologico che ha coinvolto un esperimento di incubazione su un suolo fertilizzato con N ed una parte di modellistica. Lo studio della parte generale ha evidenziato come il suolo effettivamente costituisca ancora un punto cruciale per quanto riguarda il contributo all'interno del ciclo del C all'interno degli ecosistemi forestali, la cui risposta è meditata soprattutto dalle significative eterogeneità presenti e quindi dalle scale spaziali e temporali dei processi. Nonostante questi punti critici, si è cercato di proporre uno schema concettuale che potesse organizzare le risposte microbiche e del suolo ad un aumento di N minerale riportate in letteratura e più in generale la risposta alla disponibilità di C e N di diverse qualità. In questo modello concettuale i microorganismi sono stati considerati come un singolo individuo che si comporta in modo da raggiungere una situazione ottimale, a seconda dello status energetico delle risorse presenti, prescindendo però dallo specifico meccanismo che porta ad un cambiamento della qualità di C ed N.

Una parte del lavoro ha riguardato la realizzazione di un esperimento di incubazione di un suolo forestale del Mediterraneo (quercus cerris) soggetto a fertilizzazione azotata. Per sito scelto e set up sperimentale aggiungendo la soluzione di N minerale nel tempo, l'esperimento rappresenta una novità nel panorama scientifico di questa tipologia di esperimenti. Inoltre questo studio ha permesso

di colmare il gap per quanto riguarda gli ambienti finora oggetto di studio, che hanno riguardato principalmente foreste del nord Europa e del nord America. Nonostante l'esperimento sia stato condotto in laboratorio, è stata evidenziata nel primo periodo una risposta del suolo simile alla risposta osservata in sito sperimentale coperto da quercia in Nord America ,ma soggetto a condizioni climatiche differenti, supportando l'ipotesi che la recalcitranza della sostanza organica possa effettivamente modulare la risposta del suolo all'azoto, svincolandosi dai fattori ambientali quali temperatura ed umidità. Di fatto tali fattori regolano soprattutto la composizione vegetazionale, la qualità della lettiera e la velocità con la quale viene inizialmente decomposta. Inoltre si è visto come il processo dipenda dal percorso dell'attività microbica durante l'incubazione e come questo influisca in modo non lineare il risultato finale. I risultati hanno inoltre evidenziato come l'N minerale possa essere usato come variabile chiave per controllare sul lungo termine l'attività microbica, by-passando la necessità di entrare nel merito di specifici effetti di natura propriamente biochimica (i.e. il processo di acidificazione).

E'stato quindi sviluppato un modello di decomposizione della lettiera ed accumulo della sostanza organica, partendo da uno schema del C standard. Il ciclo dell'N è stato modificato in modo da tener conto di tutte le interazioni tra i due cicli, con particolare attenzione all'N sia in forma minerale che organica. Lo scopo del lavoro è stato quello di proporre una formulazione the esprimesse il controllo dell'N minerale sulla decomposizione della sostanza organica e che potesse essere incluso negli attuali modelli dinamici del suolo. Il merito è stato quindi la modellizzazione di un processo che allo stato attuale non è ancora completamente inserito nei modelli del suolo. Il tentativo è stato quello di trovare un compromesso tra la complessità dei fenomeni coinvolti ed l'approccio modellistico che potesse mantenere una semplificazione matematica in modo da essere facilmente gestibile ed interfacciabile con altri modelli. La formulazione proposta modifica l'attività di decomposizione come funzione della domanda microbica e della disponibilità di N minerale nel suolo. Il modello, caratterizzato da un limitato numero di parametri da ottimizzare, è in grado di riprodurre molte delle evidenze sperimentali riportate in letteratura, cercando di sottolineare il ruolo dei microorganismi e dell'N minerale sull'attività di decomposizione della sostanza organica recalcitrante. Nonostante i risultati promettenti, è stato evidenziato come il modello necessita di un'ottimizzazione ad hoc in modo da poter essere esteso a tutto il profilo del suolo. Il modello proposto offre comunque una formulazione interessante che può essere inserito all'interno dei modelli dinamici attuali suolo-pianta.

La fase finale del lavoro è stata dedicata alla modifica di un paio di modelli in modo da considerare il comportamento della comunità microbica che cresce su diversi substrati, second l' "input matching principle" ed il raggiungimento di uno scopo di natura ecologica i.e. massimizzazione della crescita microbica. Il comportamento delle popolazioni è stata studiata in particolare per

animali e acquisizione di risorse a grande scale, ma rarametne è stata applicata alle dinamiche del ciclo del carbonio nel suolo. Il secondo modello, anche se è ancora un esercizio teorico, ha fornito I risultati migliori e apre quindi una nuova dimensione allo studio dei modelli di decomposizione della sostanza organica a scala di plot.

CONTENTS

1. INTRODUCTION AND OVERVIEW

Half of the carbon dioxide (CO_2) emitted in the atmosphere as C fossil-fuel combustion and tropical deforestation is stored in oceans and terrestrial biosphere, in particular soils constitute the largest C reservoir; with a total global storage of approximately 1500-2000 Gt C (about 80% of the total terrestrial C storage) holds three times as much carbon as vegetation with a C stock of about 500 Gt C and about twice as much as the atmosphere (Jacobson et al. 2000). In particular the terrestrial ecosystems at the temperate and boreal latitude have been indicated as the main sink for C (Prentice et al.2001; Rayner et al.1999; Ciais et al.1995,), with a significant contribution due to the forests. Forests at northern latitude are indicated as the main responsible for terrestrial CO_2 uptake (fig.1); stocking as 33% of the emitted C-CO_2 are likely to contribute significantly to a sink-role in the global C budget (Holland et al.1997; Bonan 2008) with the largest amount of C stored in the soil.

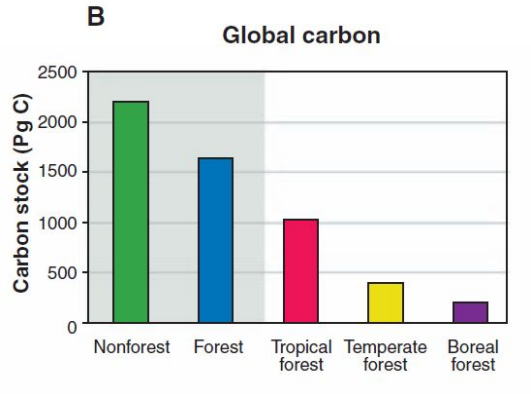

Fig.1 Total carbon stock of nonforest and forest biomes, including soil and plants (from Bonan 2008)

Soil constitutes a dynamical compartment within the biosphere interacting with atmosphere showing strong feedbacks. For instance the carbon (C) cycle is constituted by a series of processes (fig.2) that in undisturbed forests included principally the CO_2 assimilation by plants, deposition of biomass to the soil (litter fall, fine root production etc) and then the mineralization process with the release of C-CO_2 to the atmosphere regulating hence the net exchange of C between terrestrial biosphere and atmosphere (Trumbore 2006). The sequestration of atmospheric CO_2 by natural ecosystems is linked to the degree of stabilization once is fixed as biomass by vegetation. Actually each compartment, from leaf, fine root to wood production until the final C-immobilization in the soil matrix, is characterized by different turnover (i.e. 1/ MRT where MRT means “mean residence time”). Hence the soil organic carbon (SOC) pool provides the longer transient sink for the net increase of C fixed by plant and returned to soil.

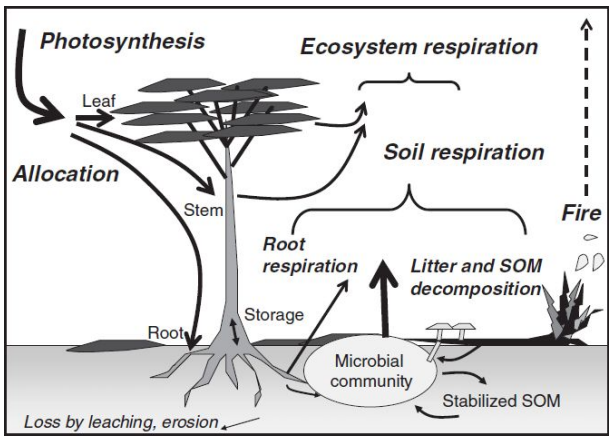

Fig.2 Pathways of carbon flow through ecosystems. (from Trumbore 2006)

However the term soil C sequestration refers to the positive balance between the input to soil of plant-fixed CO_2 via the photosynthetic pathway and the release to the atmosphere of C due to the decomposition of these residues by activity of heterotrophic microorganisms. Forest soil C pool may thus act as a source of C rather than sink of C for atmospheric CO_2 depending on the balance between the two terms against external disturbance of various origins (i.e. N depositions, fire, forest management etc).

1.1 Drivers controlling forest c sequestration

Processes of C sequestration at the ecosystems level are subject to drivers of several type, including environmental factors such as direct climate control (Davidson and Janssens 2006; Rustad et al.2001; Melillo et al.1993; Melillo et al.2002) rather than ecological drivers such as plant species, C substrate availability, nutrient supply and availability and land use change effects (Nabuurs et al.2007; Hungate et al.2003; Reich et al.2006) that can interrupt period of C uptake realising C because of disturbance or harvest. Climatic factors in turn can control shift species and vegetational composition (Dale et al.2001; Thuiller et al.2008), regulating aboveground cover and composition, and consequentially resource distribution and the response of soil due to its interface with litter quality and production (de Santo et al.2002). All this items contribute with different temporal and spatial scales to promote or reduce the potential for forests mitigation against increasing CO_2 concentration in the atmosphere. In particular nitrogen (N) availability has been indicated as a key-driver in C sequestration operating at level of C stock size in both plants and soil (Hungate et al.2003; Reich et al.2006; Magnani et al.2007; Reay et al.2008; deVries et al.2009). In this contest soil plays an important role by medium of microorganisms activity that control the nutrient cycling in the ecosystem and regulate N retention and diffusion. Soil modulates thus the availability of mineral N (Aber et al.1998) concurrently with plant competition for N uptake, allowing the aliquot remaining to leach or loss in gaseous form.

In fact plants productivity is mainly sustained by N supply by decomposition of litter and soil organic matter (SOM). On one side the indirect effect of the climate change is the increase of temperature that can promote soil C mineralization and thus potentially can enhance soil N availability (Rustad et al.2001; Melillo et al.2002). On the other side, a contribution to soil mineral N availability is external. Input of N in forest ecosystems are supported by artificial amendment for improving timber production or in undisturbed ecosystem by the anthropogenic N deposition supplies.

Presently forests ecosystems receive a significant aliquot of external N as continuous input from atmospheric wet and dry N depositions which is one of the problems associated with global change and in particular with atmospheric pollution (Galloway et al.2003, Galloway et al.2004, Nabuurs et al.2007, Aber et al.2003) highlighting the utterly importance of interactions between C and N cycle within the climate change contexts (Gruber and Galloway 2008).

After 2000 several studies underlined how external N has an impressive effect on net forest C sequestration in temperate and boreal forests in both Europe and United States (Hogberg et al.2007; Magnani et al.2007; DeVrie et al.2006; DeVrie et al.2009), starting from the work of Townsend (1996) and Aber (1998) until the work published by Magnani and co-authors on Nature (Magnani et al.2007) where it was shown the relation between wet N depositions and net ecosystem productivity (NEP) in several forest sites in the northern hemisphere (fig.3). Actually N constitutes a primarily limiting nutrient at the middle and high latitude (Vitousek et al.1998), however the direction and magnitude of the ecosystem response is still under investigation due to the highly complexity governing soil and trees C sequestration (Janssen and Luyssaert 2009; Reay et al.2008; Sutton et al.2008).

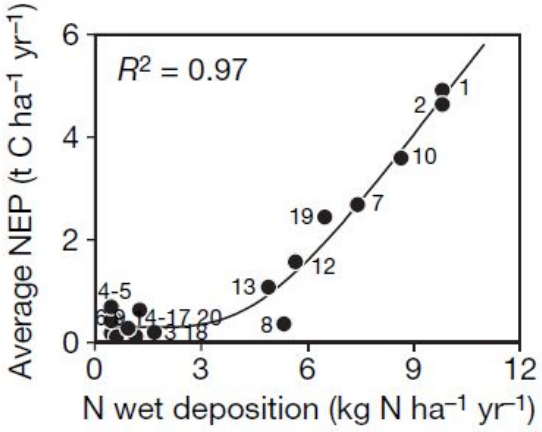

Fig.3 Average NEP related to N deposition in several forest sites. Numbers refer to site codes reported in table 1 (Magnani et al.2007).

Several field experiments with Free Air CO_2 enrichment (FACE) and chamber studies have been performed in order to investigate and understand the regulatory mechanisms of ecosystems

response. As previously mentioned soil N status has been indicated as having an important positive role in driving the processes under atmospheric CO_2 increase (Oren et al.2001; Reich et al.2006). However Hyvönen (2008) showed how N deposition not always speed up the sequestration in trees but how plant N-use efficiency were dependent on soil N status, showing a lower plant response in N-rich sites, while promoting at N-poor sites. Hence the evaluation of N fertilization efficiency as tool to improve and maintain stand-level C density and soil C stock is a necessary step.

In particular it is necessary to detect the fate of N once enters the forest ecosystem, since the pathway of applied N drives the ecosystem response in term of C sequestration. In fact the wide range of ecosystem responses may be justified by the proportion of applied N intercepted by soil or lost to drainage water rather than taken up in the aboveground vegetation. The former includes biotic and abiotic immobilization mechanisms of N in organic form in both labile (i.e. microbes) and recalcitrant form (Schulten and Schnitzer, 1998) by microbes or physical-chemical processes as enzyme-like condensation reactions.

This is a critical issue, since on one hand the major the N plant uptake and the production of woody tissues, the major the C stock due to the high C to N ratio as underlined by Nadelhoffer (1999), on the other hand the soil has a lower potential for C sequestration due to the lower C/N ratio (about 30 on average) but the lower turnover due to process of stabilization has the potential to guarantee a more stabile C sequestration on the long term. Moreover soil C stabilization is coupled to transformation of N in recalcitrant form, making N less available to plant until SOM mineralization process is performed. Thus, the effectiveness of N input to natural ecosystems toward increasing plant C stock appears to be constrained by these mechanisms that do not make N available to plant assimilation (Magill and Aber 1998; Aber et al.1998; Magill et al.1997; Davidson et al.2003).

For example at Harvard Forest LTER site, a long term fertilization experiment has been carried out since 1988 supplying mineral N at a rate of 50 and 159 Kg ha^{-1} y^{-1}. In this site a retention efficiency in the soil of 85% was detected after 6 years with a negative feedback on plant growth since a limitation on foliar and root N concentration appeared after 1995 (Magill et al. 2004). Nadelhoffer (1999) collected results from ^{15}N-tracer studies conducted in European And North American forests (short term experiment, 1-3 years) and showed that about 70% of applied ^{15}N solution was recovered mainly in the soil while only 20% in trees. In the field experiment reported by Micks (2004), a fast disappearance of N added occurred, and the N extractable value reached the pre-adding value just after few days the addition, relating thus this fast disappearance mainly to soil abiotic mechanisms. Contrarily to this evidences, Zak (2004) in a N fertilization experiment in Michigan, found that the applied 24 g ^{15}N solution was mainly recovered in plant compartment, leaves and branches, after one year since application while it was not detected a substantial amount

of labelled N in SOM or soil mineral N. The applied N to the soil was quickly recycled by microbes and thereinafter retained in plant biomass.

For instance, an other indirect effect of N deposition is to increase leaves N concentration (Magill et al.2004; Mellert et al.2004; Pregitzer et al.2008) that controls conversion of CO_2 assimilation and hence C sequestration at leaf-level. In turn altered foliar nutrient levels are likely to change nutrient fluxes with litter fall quality and amount which may affect soil mineralization process and, thus the plant-availability of N closing thus the cycle.

Actually this internal increase of N may interact directly with decomposed organic matter inhibiting microbial activity and SOM decomposition (Berg and Menteemayer 2002). For example Pregitzer (2008) showed an increase of internal N in leaves litter in four sites subject for 10 years to artificial N fertilization at rate of 30 Kg N ha^{-1} y^{-1}. The other macroscopic evidence was a net C sequestration in woody biomass and in the top soil principally. Since an increase of litter production was not detected, it is likely that external N increase (mineral N supply) and internal N (foliar N) have contributed simultaneously to increasing soil C stock due to a direct effect on soil microbes suppressing decomposition process.

Hence, concurrently to the attention moved toward plant response to increasing N availability, a lot of interest has been emerged toward the quantification of soil C sequestration with N retention (DeVrie et al.2009; Reay et al.2008; Aber et al.2003; Aber et al.1998). At the current state, N fertilization experiments performed at plot scale have been reported to promote soil C sequestration in forests at the rate ranging from 5-35 Kg C/ Kg N for low dose of N fertilizer (less than 50 Kg N ha^{-1} y^{-1}) as estimated by de Vrie and co-workers (2009) in European forests; 21 Kg C/Kg N as estimated in Nadelhoffer (1999) supposing a soil C/N of 30; 23 KgC /Kg N in Pregitzer (2008) averaging the results in 4 experimental sites; 11 Kg C/Kg N on average by Hyvönen (2008).

However, although the experimental evidence showed how soil C stocks may increase as a consequence of increased N deposition, it is still not clear the soil microbial response and if a saturation will occur.

1.1.1 Litter decomposition: the first step toward soil c sequestration

Within the contest of C cycle, litter production and quality is the main driver of potential for soil to sequester C. Markedly litter quality and soil nutrient status control substrates decomposition and formation of recalcitrant compounds. In fact the more recalcitrant litter compounds as ligno-cellulose complex, nitrogen content involved in the humification processes received particular attention by scientists starting from Berg (1987), Melillo (1982) until Chertov (2007). The process of soil C sequestration starts with litter deposition and the decomposition by macrofauna and thereinafter by heterotrophic microorganisms follow. Soil microbial community drives thus the path

of C mineralization on both short and long temporal scales, thus on humus formation until the final step is achieved where organic matter is ultimately chemically and physically stabilized within the mineral matrix.

In order to perform biomass synthesis and to cover energetic demand, microorganisms need both C and N as nutrient, the amount of is regulated by stoichiometrical laws. Hence, after physical constrains as temperature and soil moisture, N availability is the first limiting nutrient to C-rich substrates decomposition. The N effects on litter degradation have been investigated in literature showing different response depending on rate of fertilization and stage of decomposition (Knorr et al.2005; Aber and Magill 1998). It is well known that N amendment tends to increase the degradation of cellulosic litter and to retard the mass loss of lignified litter and humified litter (Fog 1988; Berg and Matzner 1997; Sinsabaugh et al.2002). The conceptual model presented by Berg and Matzner (1997) shows the different phases of litter decomposition individuating a third stage where N has a suppressive effect on degradation and hence a positive contribute on C sequestration (Fig. 4).

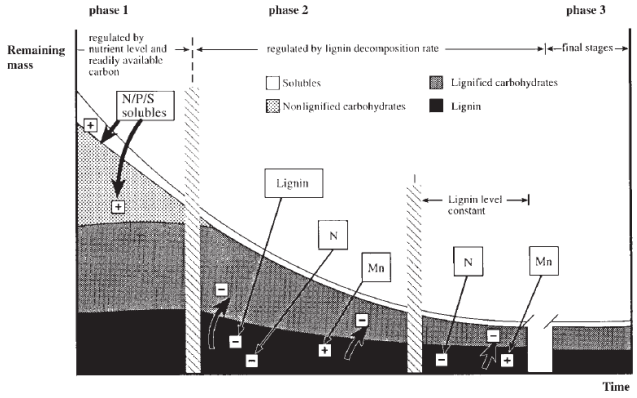

Fig. 4 Conceptual model for litter decomposition and its transformation to humus (Berg and Matzner 1997)

Thus it was underlined how the response depends on stage of litter decomposition and age of organic matter. For example N status showed a suppressive effect on respiration rates of humus (Berg and Matzner 1997), as evidenced by a lower C mineralization in soil characterized by narrow C/N ratio (Persson et al.2000). On one side, the accumulation of C and N in the late stage of decomposition is the physiological result of the humification process, on the other side the surplus of N in the soil, both in the mineral and organic form, can promote the C accumulation (Berg and Matzner 1997; Magill and Aber 1998) influencing microbial activity and thus altering the ecological functions of soil microorganisms. For instance it has been showed experimentally (see next section) that mineral N affects the overall microbial activity among the soil layers, from forest-floor to mineral soil, affecting potentially the C stock along the soil depth.

Moreover, it has been indicated how recalcitrance of C-substrates seems to have an important role in modulating the soil response to N addition and how climatic drivers have a minor role in controlling humus decomposition rate (Berg and Meentemayer 2002; Couteaux et al. 2005; Knorr et al.2005).

1.1.2 Inorganic N effects on soil c sequestration: experimental evidences from literature

In the past artificial N fertilization has been performed in order to improve timber production in several European forests. Thus the interest toward investigation on N deposition effects on forest ecological functionality started in these sites. Following, the influence of N addition on soil processes have been investigated in several forests in north Europe and north U.S. among laboratory soil incubation in order to verify any positive or negative feedback on C cycle. In particular the attention was moved toward plant growth response and soil microbial activity.

In the last 20 years incubation experiments with N fertilization have been performed on organic (close to humus stage) or mineral soils of temperate forests and field experiments have been carried out in N limited ecosystems among a climatic gradients and a different soil N-status under natural conditions (Tab. 1,2,3). Presently the area of interest covers a wide range of ecosystems from boreal to tropical forests. Every experiment was characterized by type of site, fertilizer used, rate of N fertilization and variables monitored. N was mainly added as aqueous solution rather than as pellets in several dates throughout the year, in the form of NH_4NO_3, KNO_3 or $(NH_4)_2SO_4$ depending on the nature of ambient N deposition rather than experimental choice. Rates range between 5 up to 100 Kg N ha^{-1} y^{-1} with a peak of 200 Kg N ha^{-1} y^{-1} for experiments conducted *in situ*, while N amount reached values up to 2-3 mg N g^{-1} OM in the incubation experiments. The spatial scale of observation includes principally the stand scale, while monitoring activity is performed from hourly to yearly time scales observing processes in litter rather than along soil depth. Depending on the window of observation different variables have been measured compatible with aim of the study, complexity and financial support.

Conventional measurements *in situ* are constituted by C and N pools size when performing long term fertilized experiments or mass loss measurements in the case of litterbag studies. C fluxes are measured with both the Eddy-Covariance method at ecosystem scale rather than soil static and dynamic chambers at plot scale. For instance soil respiration is more sensitive to external disturbance if compared to SOC measurements that require a huge sampling and a long term experiment in order to detect a significant change (Conant et al.2003). While in the field soil respiration includes several contributions (Ryan and Law.2005), in laboratory incubation the C-mineralization has been used as a measure of the microbial activity. Since all majority of C and N

processes in soil are driven by heterotrophic microorganisms, a lot of studies have been performed in laboratory in order to have a robust evaluation of the microbial response to N fertilization, although the C supply by litterfall and interaction with plants is missing. However laboratory work allows us to investigate better the biochemical response of microorganisms. In fact C-mineralization is the results of decomposition of organic matter in order to acquire energy and matter for synthesis of biomass, enzymes and other bioproducts. Bulk soil respiration can be then coupled to measurement of microbial biomass rather than community structure among other biological variables such as ATP level (Dilly and Nannipieri 2001). A further method in order to detect the activity at process level in both field and laboratory incubation is the study of enzymatic rates.

Indeed soil microbes are mainly heterotrophic and enzymes are important catalysts of the most fundamental processes involving organic C and nutrient dynamics (Nannipieri et al.2003; Caldwell 2005). These mediators operate at the level of C-polymers and constitute hence the first step toward the formation of small molecules (i.e. glucose) available for microbial *iter* that includes uptake, assimilation, biosynthesis and to meet the cell energetic demand. The critical issues moved by the measurement of enzymatic rates concerns the difficulties to distinguish between intracellular and extracellular enzymes, rather than the activity of enzymes absorbed on the mineral matrix. However, it has been shown as C-enzymes can be correlated with litter decomposition rates of different constituents (Sinsabaugh and Moorhead 2000) as reported in fig. 5. Moreover soil enzymatic activity is very sensitive to both natural and anthropogenic disturbances, and shows a quick response to the induced changes.

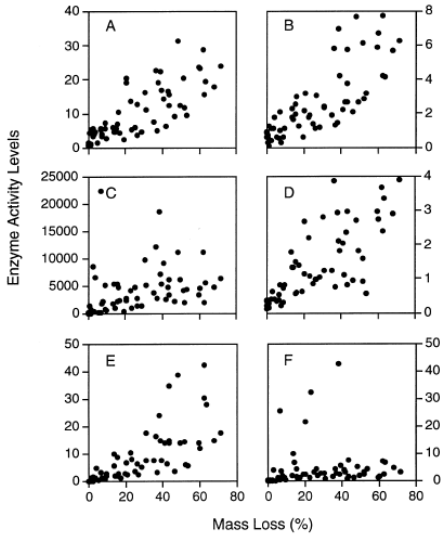

Fig. 5 Activity levels of enzymes (units vary) associated with birch wood decay in northern New York, USA. A. glucosidase (mmolgAFDM^{-1} h^{-1}), (B) xylosidase (mmolgAFDM^{-1} h^{-1}), (C) endocellulase (unit gAFDM^{-1} h^{-1}), (D) exocellulase (mmol gAFDM^{-1} h^{-1}), (E) phenol oxidase (mmolgAFDM^{-1} h^{-1}), (F) peroxidase (mmolgAFDM^{-1} h^{-1}). From Sinsabaugh and Moorhead 2000.

For these reasons the role of soil exocellular enzymatic production has gained with time more interest from the scientific community (Sinsabaugh and Moorhead 1994; Barraclough, 1997;

Moorhead and Sinsabaugh 2000; Schimel and Weintraub 2003; Schimel and Bennet 2004) and it became a really complementary tool for investigating microbial physiological status in both laboratory and field.

With regards to the N anthropogenic effects in natural ecosystems, microbial enzymes have been widely studied in litter and soils among different natural sites, with particular attention to temperate forests. At this point is of importance to make a distinction between different enzymatic families, depending on the reaction catalyzed rather than the type of donor. In laboratory the enzymatic assays allow to distinguish the activity rate of a particular enzyme depending on the choice of the simple artificial substrates that are supplied to microorganisms. In particular it is possible to cluster enzymes in hydrolytic enzymes that during decomposition of particular a C-substrate returns smaller compounds. Enzymes belonging to this class are for example enzymes devoted to degradation of labile C-polymeric compounds. It includes hence enzymes such as α-glucosidase, β-glucosidase, mainly produced by fungi (Miller et al.1998) and responsible of the glucose release, Cellulase and Xylanase devoted particularly to cellulose decomposition.

An other important class of enzymes is the oxidase that uses oxygen atoms as the electron acceptor. Within this class, phenol oxidase belongs to the soil enzymes of interest in the context of C cycle. In fact polyphenol oxidases are enzymes important for degradation of recalcitrant substances such as lignin and are involved in the formation of humic substances from their monomeric precursors. Oxidase family is referred sometimes, in soil scientific papers, as polyphenol oxidase (PPO), tyrosinase or laccase. Actually the distinction within this group and the exact chemical reaction involved is still far from a complete understanding due to the several processes catalyzed, the reactions can be nonspecific or can deliver several different products. Usually tyrosinase (i.e. chatecoloxidase), is the model for phenol oxidase enzymes, but it is difficult to determine the specific activity within the broad range of enzymes. Laccases share a high number of donors with tyrosinase (Baldrian 2006; Tuncer et al.2004), being able to catalize both o- and p-diphenols, while tyrosinases oxide diphenols and monophenols in particular. Laccases are mainly found in fungi involved in the degradation of wood (white-root fungi and saprotrophic fungi) although it has been found widespread in bacteria too; tyrosinases are active in both fungi and bacteria (Claus 2004; Baldrian 2006; Burton 2003). Peroxidase enzymes are included as well in the overall soil oxidative activity but we will give less attention in this work. The critical issue moved toward oxidative activity is the capability of these enzymes to catalyze both polymerization and depolymerization processes, hence depending on the balance of the two reactions loss or storage of organic matter can occur (Berg and Matzner 1997).

Among enzymes involved in C-cycle, other enzymes exist interesting N and phosphorous (P) cycle differentiating on the basis of the organic compound attacked, i.e. N-acetyl glucosaminidase is

involved in degradation of chitin (Miller et al.1998; Olander and Vitousek 2000) while acid-phosphatase is a crucial enzyme involved in organic P transformation.

1.1.3 Experimental results

In the following table (Tab.1,2,3) some of the main results reported in literature with the measured variables and observed effects have been collected. We reported mainly long term N fertilized experiments conducted *in situ* or works involving degradation of litter on the long temporal scale (at least after 2 years or more since starting decomposition). We did not take in to account studies performing dissolved organic C (DOC) measurements, although is an other important aspect of C stabilization/mobilization interested by N addition (Sinsabaugh et al. 2004; Waldrop and Zack 2006; Demoling et al. 2008; Magill and Aber, 2000). The difficulties to analyze conceptually the DOC measurements are related to the nature of this mobile C fraction, that in turn depends on the analytical methodology utilized for its quantification. For instance DOC compounds can include low molecular weight polyphenols and carbohydrates, thus both labile and recalcitrant part, supporting differently the energetic microbial demand and referring to different processes affected within soil.

Reference	Ecosystem/soil	N treatment	Experimental evidence	Note
FIELD				
Allison et al.2009	boreal forest	since 2002, 100-200 Kg N ha^{-1} y^{-1}	C/N microbes decreased, oxidative activity decreased in some monitoring dates	no changes in soil respiration were detected
Bowden et al.2004	temperate Forest (Harward site)	10-15 Kg N ha^{-1} y^{-1}	Suppressed total soil respiration	
Butnor et al.2003	temperate forest (Duke site)	112 Kg N ha-1 y^{-1}	Suppressed total soil respiration	
Demoling et al.2008	coniferous forest soil, Norway	since 1987, 100 kg N ha^{-1} on one site, 50-100 kg N ha^{-1} in other two sites	N tended to decrease bacterial growth rate, reduction of microbial biomass, decreased fungal biomass	
De Vries et al.2006	European Forests, from plot scale to european scale	N deposition 1960-2000	SOC increase	
Franklin et al.2003	pine forest, Norway	since 1971	Modelled SOC accumulation from ^{14}C measurements	70% of increased SOC estimated to be from decreased decomposition rate and 20% was attributed to increased litter production
Hyvönen et al.2008	pine forests, Sweden and Finland	30 - 200 Kg N ha^{-1} y^{-1}	SOC increase	SOC increased even when plants did not respond to N addition
Knorr et al.2005	Forest, tundra and grassland ecosystem	different N fertilization rates or N deposition amongs litters and sites	litter decomposition is inhibited at sites where fertilization rates were 2–20 times the anthropogenic N deposition, or low level ambient N deposition (5-10 kg ha^{-1} yr^{-1}) or for low quality litters	
Mäkipää R. 1995	boreal fores	5-7 times over a 30 years period (total 596-926 Kg N ha^{-1})	Surface SOC increase	

Magill et al.2004	temperate Forest (Harward site)	5 -15 Kg N ha^{-1} y^{-1}	No change detected in SOC	
Magnani et al.2007	Temperate and Boreal forests	Current N wet deposition	increased Net Ecosystem Productivity (lower Ecosystem respiration)	
Magill and Aber 1998	temperate forests (Harward site)	15-30 Kg N ha^{-1} y^{-1}	Litter decomposition decrease on late stage of decomposition	
Mo et al.2008	tropical forest (south China)	one year of N fertilization, 5-10-15 Kg N ha^{-1} y^{-1}	total soil respiration declined in the highest N treatment	
Nöhrsted et al.1988	boreal forest	one N fertilization, 150 and 600 Kg N ha^{-1}	lower total soil respiration	
Olson et al.2005	boreal forest	100 Kg N ha-1 y^{-1}	lower total soil respiration	
Pregitzer et al.2008	temperate forest (Michigan)	30 Kg N ha-1 y^{-1}	Surface SOC increase	SOC increases due to decreased decomposition rate rather than increased detrital inputs
Prescott 1995	pine litter	3 y; N fertilization	no effect although the average mass remaining was slighlty larger in the fertilized plot	
Sodestrom et al.1983	boreal forest	added 150 Kg N ha^{-1}	lower soil respiration, lower microbial biomass	
Thirukkumaran and Parkinson 2002	Canadian pine Forest	added 188 Kg N ha^{-1}	No effects on microbial variables	soil respiration not affected after 1.5 y of fertilization

Tab 1. Literature Studies performed in the field, investigating the effect of mineral N addition. SOC= soil organic carbon.

Reference	Ecosystem/soil	N treatment	Experimental evidence	Note
LABORATORY				
Allison and Vitousek 2005	tropical forest (Hawaii)	28 d incubation; added simple and complex C and nutrients (N,P), 1.6 mg N g^{-1} soil,	lower respiration when addition of ammonium only, responded positively when labile C were added with N-compounds	
Blagodatskiy et al. 1993	gray forest	28 d incubation; added N solutions at a rate of 33.6 μg N g $soil^{-1}$	C mineralization decrease	
Craine et al.2007	grasses, forbs and woody plants	218 d incubation; N fertilizer, soil and litter incubation	C mineralisation decrease	N limitation increased decomposition of SOM (recalcitrant C)
Gutierrez et al.2008	evergreen oak litter from a Mediterranean forest	72 hours incubation; added N solution, 0.21, 2.1,10.6, 21.2 gN l^{-1} of litter water solution	N suppressed microbial activity in the litter layer depending on litter quality and amount of N. Lower respiration and lower PO at the highest N dose.	
Hagedorn et al.2003	mesocosmo study, model forest with open-top chambers	4 y experiment; added 6-7 Kg N ha^{-1} y^{-1} and 60-70 Kg N ha^{-1} y^{-1}	retard of old and humified SOC decomposition	
Green 1995	agricultural soil, corn residue decomposition plus inorganic N	90 d incubation; N added at a rate of 0,11,22,44 μg N g $soil^{-1}$	C mineralization decrease in control soil (without residues) for highest N dose	
Recous et al.1995	agricultural soil, maize residues plus mineral N	125 d incubation; added N solution in order to have an initial concentration of 10,30,60,80 and 100 μg N g^{-1} soil	C mineralization decrease in control soil (without straw) for highest N dose	

Sjoberg and Persson 1998	Forest, intact soil humus cores	160 d incubation; addition of 4,20, 100 μg N g^{-1} SOM (equivalent to about 0.1,0.6 and 3 Kg N ha^{-1})	C mineralization not affected	higher heterogeneity between soil samples
Sodestrom et al.1983	Boreal forests	22,45 and 175 d incubation; added N as solution, 2 mg N g wet-soil $^{-1}$	C mineralization and microbial biomass declined	
Thirukkumaran and Parkinson 2000	Canadian forest, pine forest floor, humus sample	120 d incubation; added the equivalent of 0-188-300 kg N ha^{-1}	lower microbial basal respiration and metabolic quotient	

Tab 2. Literature Studies performed in the laboratory, investigating the effect of mineral N addition on microbial activity. SOC= soil organic carbon

Reference	Ecosystem/soil	N treatment	Experimental evidence	SOC/LITTER MASS
Saiya-Cork et al.2002	temperate forest (Michigan)	30 Kg N ha^{-1} y^{-1}	PO increased in litter, decreased in the soil	increased litter decomposition and declined decomposition of SOM
Waldrop et al.2004	temperate forest (Michigan)	80 Kg N ha^{-1} y^{-1}	PO activity declined in high-lignin litter ecosystem	SOC increased in high lignified litter ecosystem
Gallo et al.2004	temperate forest (lower Michigan)	30 and 80 Kg N ha^{-1} y^{-1}	PO activity declined and glucoxidase activity increased in soil	
Sinsabaugh et al.2005	temperate forest (northern Michigan)	30 and 80 Kg N ha^{-1} y^{-1}	In mineral soil activities toward acquisition of labile C increased while PO activities declined, in high-lignin litter ecosystems	SOM decreased in low-lignin litter ecosystem, while increased in high-lignin litter ecosystems
Sinsabaguh et al.2002	forest, mixed deciduous (NY)	20-80 Kg N ha^{-1} y^{-1}	shift EA away from N acquisition and from PO activity and toward polysaccharide hydrolisis. Effect of added N more pronounced in the later stage of decomposition when PO activity become more prominent	lower litter mass loss in high-lignin litter
Carreiro et al.2000	forest, mixed deciduous (NY)	20-80 Kg N ha^{-1} y^{-1}	Cellulolityc activity increased, PO activity declined for low-quality litter	lower litter mass loss
DeForest et al.2004	temperate forest (Michigan)	30 Kg N ha^{-1} y^{-1}	reduced microbial activity and biomass, declined PO in litter	no alteration of microbial community structure
Henriksen and Breland 1999	agricultural soil, wheat straw plus inorganic N	70 d incubation; addition of 2.1, 8.6, 16.1, 32.2 mg N g added C^{-1}, correspondent to 0.02-0.3 mg N g $dwsoil^{-1}$	At the highest N content, significantly lower C-hydrolitic enzymes	C mineralization decreased during incubation with N
Michel and Matzner 2003	temperate forest soil (Germany)	15 weeks incubation; up to ten times the initial concentration of mineral N, from 3 μg N $gSOM^{-1}$ to 3 mg N $gSOM^{-1}$	EA is related to C/N ratio of organic layer in the late stage of decomposition, C-hydrolizing enzymes were affected in N-poor soils; no effect on PO and NAG	trend toward higher metabolic quotient, in some samples

Tab 3. Literature Studies performed in the field or in laboratory, investigating the effect of mineral N addition on microbial activity performer studying enzymatic activity. SOC, soil organic carbon; EA, enzymatic activity; PO, oxidative activity.

Several laboratory and field experiments have shown a trend toward a soil C sink and a suppressive effect on microbial activity (see review of Berg and Matzner 1997, Reay et al.2008) affecting microbial functionality and structure (i.e. fraction of bacteria on fungi) not only in litter but in humus and mineral soil as well.

Evidence for changes in soil C stock under mineral N enrichment comes from a variety of sources, including changes in soil respiration/carbon mineralization rates in the laboratory incubations or field. Other studies report changes in litter mass accumulation and variations in soil organic C pools.

In the field the increase of SOC was mainly due to the combination of promoted litter input and decrease of SOM decomposition with a significant contribution of the latter. N-induced inhibitions on soil processes have been investigated with a model based on the continuous-quality decomposition theory (Agreen and Bosatta 2001,) and then coupled to a plant growth model and applied at ecosystem scale by Franklyn (2003), optimizing the model parameters on ^{14}C data and other field data. The authors investigated the main determinant of mechanisms responsible of soil decomposition modifications. They underlined how the fundamental factors responding to N amendment were related to litter characteristics (initial litter quality, decomposition rate) and microbial community functionality (higher microbial efficiency, lower microbial growth rate).

In fact in some cases soil responded negatively when plants did not respond to N amendment. This lead to an unbalance between litter input and SOM decomposition, preventing the soil C to reach a steady state.

Concerning the direct effect of mineral N on soil microbial biomass and activity, the result was a reduction in enzymatic rates, microbial biomass and respiration decline with lowering soil C/N ratio in both field and laboratory experiments. Specially, N has been indicated having a mixed effect on soil enzymatic activity depending on the enzymatic family involved, conducting mainly to an uncoupling of degradation activity toward labile C and recalcitrant C, expressing a declining enzymatic activity of the oxidative rates and shifting enzymatic activity away from N acquisition. Downregulation of oxidative activity by N availability has occurred in several field experiments It was reported how the response is site specific and depends on the dominant vegetation, thus the general scheme reports that plants with more lignified litter lead to a negative response under N fertilization compared to site characterized by litter with a higher quality, stimulating cellulose degradation and hence alleviating N limitation (Waldrop et al.2004; Sinsabaugh et al.2002; Sinsabaugh et al.2005; Carreiro et al.2000). It is important to keep in mind how oxidative enzymes represent two side of the same process under a chemical point of view. On one side depolymerization occurs, on the other side polymerization occurs. Concurrently in litter decomposition, the cellulase activity can move the C balance toward a higher mass loss depending

on the litter quality. However, in humus and mineral soil, oxidative activity decline have been reported coupled to an increase of SOM sequestration.

1.1.4 Mechanisms proposed in literature

Several biochemical hypotheses have been proposed, investigated and partially verified in order to explain the mineral N effects on microbial activity.

1. C-LIMITATION (effects on C-quality)

Since soil processes are mainly mediated by heterotrophic microorganisms, higher C-recalcitrance makes microbes more C-limited affecting degradation efficiency (Demoling et al.2008; Micks et al.2004; Berg et al.1998). C-substrates became more recalcitrant under increasing N availability due to processes of condensation between phenols, produced during lignin decomposition, and N compounds (mineral N, amino acids, amino compounds etc) *via* both abiotic and biotic reaction involving enzymes or enzyme-like reaction (Fog 1988; Berg et al.1998; Aber 2000, Moran et al.2005; Swanston et al.2004) or complexation phenomena of phenols (Davidson et al.2000). Moreover the degradation process of old organic matter requires a lot of energy to be processes, thus the lack of labile C to support the microbial work makes the microorganism stressed and less efficient (lower growth rate), thus N addition may aggravate the C-limitation condition.
However Sjöberg (2004), did not found any changes in the structure of lignin in pine litter incubation experiment to support this hypothesis, and proposed that N would affect directly microbial community; reporting how these condensation reactions would need a basic environment to be supported.

2. EFFECTS ON MICROBIAL COMMUNITY STRUCTURE AND FUNCTIONS

2.1 reduction of oxidative activity in lignolitic microorganisms ("ammonium metabolite repression") *via* declined enzymatic expression and/or microorganisms abundance reduction, in particular in the more lignified litter ecosystems (For 1988; Carreiro et al.2000; Sinsabaugh et al.2005). N fertilization effects include a modification of microbial community composition, in particular shift of composition toward a resistant and higher bacterial community (Demoling et al.2008; Frey et al. 2004) and higher N requiring microorganisms. Fungi (i.e. lignin degrading basidiomycetes) are the most sensitive microorganisms reported to respond negatively to N fertilization, however repression of fungal abundance rather than activity was not detected in

litter where the most important signal was the cellulase. Although the role of white-rot basidiomycetes is well studied in literature with regard to the degradation of lignin there are other functional groups involved in wood and lignin decay such as saprophytic microorganisms (i.e. actinobacteria) although with less efficiency. Hence N contribution to loss or promotion of oxidative activity occurred in ecosystems that vary widely in fungal abundance (Saiya-Crok et al.2002; deForest et al.2004; Waldrop et al. 2004; Gallo et al.2004). Blackwood (2007) ultimately indicated how the site specificity of response to N fertilization was likely to depend mainly on microbial community resident in to the soil rather than microorganism growing in the forest floor, and how the effect may operate at genetic level of enzymatic expression. Actually in more than one experiment, oxidative rates changed although no changes in fungal mass or structure occurred (Blackwood et al.2007; Hofmockel et al.2007; deForest et al.2004).

2.2 N surplus affects the chemical soil environment. It is related to the process of soil acidification due to increase N-ions that can counteract microbial activity expression. For example a depression of C-mineralization was observed in both the presence of ammonium ions (Othonen et al.1991; Allison and Vitousek 2005) and nitrates. Sinsabaguh (2008) reported how the oxidative activity is more favoured in basic environment while inhibited in acid soil. However, a negative soil response in terms of respiration and enzymatic activity was observed in fertilized soils originally characterized by a low pH (Magill et al.1997; Tirukkumaran et al 2000); in incubation experiments a suppression mechanism was detected even after the chemical effect was reduced (Södeström et al.1983); in the experiment reported in chapter 2 of the present work, different microbial responses were observed for similar pH. These evidences suggested that an acidification process can not explain completely the response of microorganisms. N osmotic stress due to the higher ionic strength have been reported in particular in experiments where N was supplied in salt form (i.e. ammonium nitrate), however, in the soil the highly reactivity of N and the several biochemical processes in the micro-sites, could confound these effects changing locally the chemical condition of environment.

2.3 N availability regulates C-substrate utilization capability hence the control on C pathway. In particular several authors indicated a shift of microbial community toward microorganisms characterized by a higher efficiency expressed as unit of C assimilated per unit of C degraded because of a higher nutrient availability (Ågreen and Bosatta 2001; Micks et al.2004; Manzoni et al.2008). Analogously Schimel and Weintraub (2003) suggested that N availability drives C pathway from catabolic to anabolic ways, hence from energy spilling to biomass and enzymatic production increasing microbial efficiency. Thirukkumaran and Parkinson (2002) proposed that

N availability promotes a shift in the "ecological efficiency" as a result of the decrease of the labile-C pool (Södeström et al.1983), hence the result of the microbial succession over different substrate qualities that sustained the establishment of a K-strategist population with a higher efficiency. Contrarily Waldrop (2004) underlined how a higher recalcitrance condition should lead to a situation of microbial stress, indicated by a higher metabolic quotient, hence by a lower efficiency.

3 EFFECTS ON MICROBIAL COMMUNITY and INTERACTION WITH C SUBSTRATES

Soil nutrient status as well labile C supply have been indicated to influence the microbial activity affecting the substrates utilization pattern (Hagedorn et al., 2003; Craine et al., 2007; Lagomarsino et al., 2007; Blagodastakaya and Kuzyakov, 2008), a mechanism referred as "preferential substrate utilization". Moreover the quality of C-substrates in the presence of N as nutrient drives the allocation of C resources in terms of substrates choice and sequentially the investment in biomass rather than enzymes following economic rules (Allison and Vitouseck 2005).

For instance some authors suggested that abundant labile C and nutrients inhibit the decomposition of the more recalcitrant C while the opposite occurs whit low N in order to gain nutrients from the N-rich pool of SOM (Craine et al.2007; Fontaine et al.2003,2004; Blagodastakaya and Kuzyakov, 2008), a process that has been called "mining". The lowering of native organic matter decomposition is a process similar to a negative priming, when a shift of microbes away from recalcitrant C occurs. This shift could also be coupled to the lower oxidative activity and higher C-hydrolytic enzymatic rates (see Chapter 2 of this work).

Although on different temporal scale and with different type of substrates, Allison and Vitousek (2005) showed how when labile resources are present, the production of enzymes for degradation of more recalcitrant substrates may be reduced. In the same experiment respiration was suppressed when adding ammonium only. Concurrently, adding labile-C alone or in combination with other nutrients, microbial cumulative respiration responded positively, as it was observed in fertilized cellulosic litter (Sinsabaugh et al.2005) rather than in laboratory where residues were incubated with mineral N (Green et al 1995; Rescous et al.1995). The type of response in the presence of labile-C could be due to the activation of a particular microbial family. Actually nutrient availability could not only regulate C-pathway but may act as a regulator for species selection (Fontaine et al., 2003; Fontaine and Barot 2005; Sinsabaugh et al., 2008) supporting the competition between different microbial families growing on labile C (i.e. litter) rather than recalcitrant-C (native SOM). Thus significant amounts of N may mediate the competition between r versus k strategist promoting the first one growing on the labile substrates (Fountaine et al.2003; Fontaine and Barot 2005).

Finally the net C accumulation is the balance between the increased labile C degradation and the lowered degradation of native SOM.

1.1.5 A soil conceptual scheme

When trying to summarize the exposed proposed mechanisms the following framework can be returned (Fig. 3). We decided to focus more on the effects of changed soil C and N fractions amount and quality on the microbial activity rather than the real mechanisms influencing the litter and SOM chemistry.

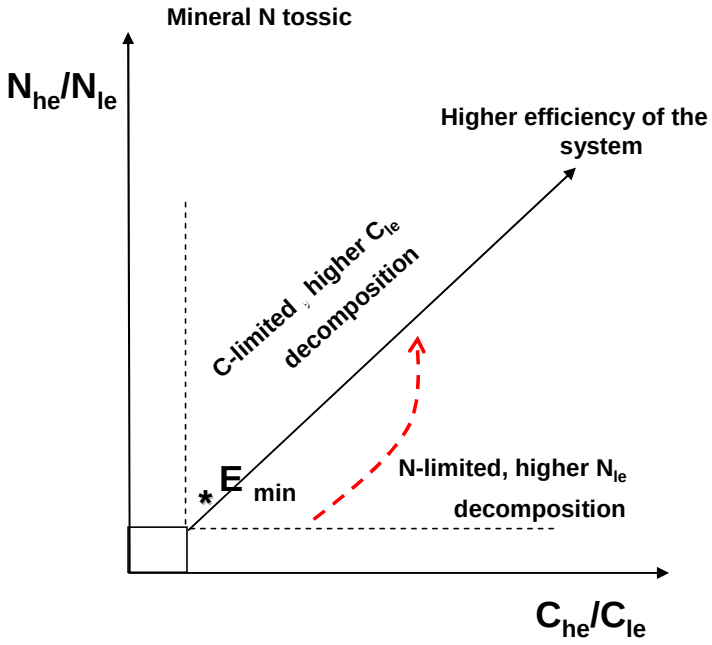

Fig. 3 Conceptual scheme of the soil qualitative behaviour in the presence of different source of C and N showing different quality.

We decided hence to cluster C and N compounds depending on their energetic level and in particular the return of C used for anabolic processes at the net of the cost, thus considering the amount of C that has to be expended. This aliquot of C is expulsed from the system as CO_2 because of catabolic processes and maintenance respiration. It was not considered any differences between what is formally called litter, cellulose, lignin, humus etc, but simply the quality of the several compounds. In this way we can indirectly include the effects due to climate change such as shift species composition on litter quality, N deposition on SOM recalcitrance, and effects due to soil texture such as clay protection etc. All these factors operate to change the capability of C-resources to be degraded by microorganisms. We do not take into account the steps within the decomposition *iter*, from assimilation to conversion in to biological constituens, i.e. microbial tissue, proteins, enzymes etc. Under an ecological point of view we can indicate the microbial goal as the maximization of the growth that can be related to the concept of fitness and the achievement of a

highly efficient condition. Markedly we could extend this concept from the single microbial cell to the whole microbial community considered as a single organism, in particular a self-organized system within soil texture (Young et al.2004) working for an “optimal physical and chemical environment" in order to achieve a more suitable environmental conditions. Hence C_{he} and N_{he} constitute C and N in form that returns a high amount of energy and matter that can be included in several biosynthetic pathways. Analogously C_{le} and N_{le} are substances that need to be supported by a higher energetic cost in order to become useful for microbial needs and that return a low yield. When we want to give an explicit form to these items, C_{he} and N_{he} can be clustered in two classes. The first one includes the substances that under a chemical point of view need a low amount of energy to be degraded (i.e. simplex C compounds, glucose, amino-acids etc), that can be easily assimilated because reduced to low molecular weight compounds and that exhibit a high microbial yield coefficient.

N_{he} includes mineral N as well, in particular ammonium and low molecular weight compounds, i.e. amino-compounds. Indeed it has been shown that microbes are able to absorb N in organic form as small molecular weight compounds, these substrates can be thereinafter used directly for building microbial tissue or proteins or deaminated internally (Barraclough 1997; Roberts et al.2009). In this class the substances that are bio-available to biomass, thus compounds that are easily degraded because easily accessible to microbial attack, are included. Analogously compounds with lower efficiency may include substrates that although with a high internal energy are not accessible to microorganisms because physically protected by mineral matrix, stabilized on fine minerals. Namely soil texture constitutes an important factor influencing the pattern within the proposed conceptual scheme. Alternatively compounds with a high energetic cost to be decomposed, thus with a significant chemical recalcitrance or because become more chemically stabilized by interaction with mineral N or amino-compounds, belong to the second class.

E* constitutes the minimum amount of labile C and labile N in order to promote the production of enzymes devoted to the degradation of the other simplex compounds rather than complex substances (Allison and Vitousek 2005). In condition of high N_{he} compounds and low C and thus C-limitation situation, the promotion of degradation of complex C occurs in order to gain more C for microbial need, coupled to a stress condition. When N_{he} in the form of mineral N increases significantly the system moves toward a situation of induced metabolite-inhibition, i.e. surplus of mineral N becomes toxic. The microbial activity is counteracted and the microorganisms are negatively affected in term of performances and growth. Analogously, when a significant amount of C_{he} is persistent in the system compared to nutrients, microbial activity is N limited. This case refers to the studies were labile C or high energetic compounds are added to soil, i.e. glucose, wheat straw etc (Chiellini et al.2007; Rescous et al.2005; Green et al.2005) but the fixation of significant

amounts of carbon as microbial biomass is not allowed, while a significant amount is converted to CO_2. Moreover part of the C-mineralized comes from both microbes and from old organic C or native SOM, called this extra mineralized C as the priming effect. Actually part of added substrates feed microorganisms growing on native substrates or can help energetically microbes decomposing complex substances and to obtain N from recalcitrant pool. When trying to explain what happen to the microbial community with regards to issues of ecological nature, the coexistence of two microbial families growing on the two different substrates and limited by different resources can be indicated (Fontaine et al.2004; Fontaine and Barot, 2005; Cherif and Loreau, 2007). For instance the formation of new bacterial specie, rather than the viability of a r-strategist community may be supposed. It is important to keep in mind that microorganisms growing faster on newly fresh and energy rich substrates were coupled to a higher turnover, thus what is quickly transformed in microbial biomass is sudden released again and fast mineralized (Chiellini et al.2007). This in turn depends on the amount of labile C added and on the activated competition between autochthonous versus zymogeneous microorganisms characterized by different growth rates, yield coefficients and turnover rates (Fontaine and Barot 2005; Chiellini et al.,2007).

When labile N is available in the system among labile C, the system moves along the dotted arrow in the conceptual scheme; the overall effect returns a microbial community more efficient, and independently if growing on C_{he} or C_{le}, the conversion of carbon to biomass rather than to other products, is higher because low energy has to be expended in order to acquire N. This statement finds support in the experiments performed by Fontaine et al. (2004) and by the work of Manzoni et al. (2008).

On the other hand, it may be possible to justify the behaviour as the results of more microbial species not competing for resources but interested by co-metabolism and mutual positive interactions. The results is hence a community involving internal feedbacks in order to increase the efficiency of the whole community as an apparent effect rather than a real efficiency change due to the internal microbial cell regulation or species selection. Hence a shift of microbial community toward more suitable substances my be indicated in the presence of labile resources, inducing a lower C-loss from the native SOM and thus an accumulation of the more stabilized soil C pool (negative priming effect, Blagodatskaya and Kuzyakov, 2008, Fontaine and Barot, 2005).

If on one hand the addition of newly C substrates can induce a significant C-loss from the soil due to the priming effect or can promote the colonization of fast growing microorganisms with a higher turnover and a higher C-mineralization, on the other hand the extend of the extra-release of C-mineralized depends on the soil nutrient status and the degree of stabilization of C and N compounds on mineral matrix and may be thus balanced by a higher C retention due to a higher yield.

Finally the capability to move along the direction toward higher microbial community efficiency depends on the microbial ability to adapt to the new condition depending on the temporal scale of the external disturbance.

1.1.5 Conclusions

N fertilization may ultimately affect the net exchange in forest ecosystems, driving C sequestration in both plants and soil influencing at macroscopic level net ecosystem productivity (NEP), plant growth and heterotrophic soil respiration. If experimental evidences and forest inventories support the important role of forests to sequester C under increasing N availability and the potential for mitigating the increasing CO_2 concentration, the role of soil as C sink and as mediator of plant response is less clear. Following the several aspects underlined in the present section appears how it would be of paramount importance to trace C and N through the forests ecosystem under N fertilization in order to restrict the uncertainties of the evaluation of the N effects on the C sequestration. Ecosystem controls include C and N pathways in both soil and plants, that in turn control the input of C to the soil (aboveground and belowground litter) and the activity of soil microbial community, ultimately the main responsible of soil transformation and stabilization. Unfortunately soil processes and long term changes in the C pools account for the most significant unknown in the C and N cycles since the effects of N on soil capability to sequester C and N in recalcitrant form are more complex due to the highly spatial heterogeneity and temporal variability. At the current state several mechanisms have been proposed in order to explain the biochemical response to increasing N, but although a general scheme may be achieved claiming explanations of energetic and efficiency nature, the real mechanisms and the temporal scale of occurrence are still far from a complete understanding. A step forward coupling experimental data, conceptual and mathematical models is of importance in order to overcome the complexity of the soil system.

1.2 Relevance of models

1.2.1 Soil models

Among field and laboratory experimental data, models are the most useful tool for data discrimination and interpretation, underlying mechanisms important in natural processes in order to organize the confusion often returned by ecological data, offering sometime a more robust scheme. Thus, trying to individuate a consistent and general scheme for process explanation, models allow to be used as a predictive tool. For instance dynamic models have the merit to try to evaluate the several feedbacks between the conceptual compartments, difficult to be investigated experimentally. For example it can be addressed for evaluating the time evolution of biological processes in the soil and in the plant system as response to external disturbance (i.e. N deposition), or used to determine the time required to recovery from the disturbance and to achieve a new steady state.

The soil compartment represents a challenge in the land surface models, because of the difficulties in providing data of belowground systems and processes (i.e. size of C and N pool, C and N fluxes, root growth, water flow etc) and due to the highly heterogeneity of spatial and temporal scales involved. However modelling represents the suitable way to deal with missing data and complex systems (Romanya et al, 2000), achieving the formulation of soil schemes that can operate at several scales with a really limited number of parameters and input without loosing necessarily the most important information at process level.

In literature several soil C-N models (coupled or uncoupled to plant production module) exist working on different spatial and temporal scale (Manzoni and Porporato 2009; Shibu et al.2006) differing in conceptualization and mathematical formulation. Most of the soil models working at large spatial scale are based on the characterization of different soil fractions with several degrees of complexity (litter, microbes, humus etc) depending on their chemical recalcitrance (Fig.4).

These pools are represented with different turnover rate, the degradation of which is expressed as a first order kinetic. The rates of decomposition depend on several factors of biological, physical and chemical nature such as temperature, soil moisture, texture usually considered as multiplicative effect, quality of substrate (C/N ratio and chemistry characteristics) and amount of substrates.

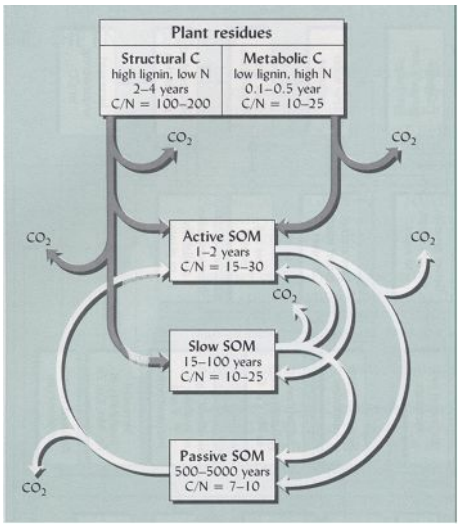

Fig. 4 Basic conceptualization of soil found in several soil models; different soil C pools with residence time and C/N ratio, derived from plant organic residues are reported, Brady and Weil, 1999.

Models such as Rothamsted (Coleman and Jenkinson, 1996) and the Century model proposed by Parton (Parton et al.1987; Parton et al.1988; Parton et al.1993) allow to performance studies at really large scale and long temporal scale allowing the results from the regional validation studies to be extrapolated to a global scale. Actually, Century, the facto soil model in literature, has been widely applied from microcosm scale (Kirschbaum et al.2002) to global scale (Schimel et al.1994).
In a recent work I collaborated with Chiti and other authors (Chiti et al. to be submitted) applying the Century model, version 4.5, to perform soil C stock changes in six italian eddy covariance flux tower sites (Mediterranean and Alpine forests) under current climate change during the commitment period of the Kyoto Protocol (2008-2012 and 2013-2017). Although the Century model has been developed primarily as a soil model for grassland systems with a simplified module for vegetation growth, it has been used with good results for other type of ecosystems including forests. One of the merits of the model is the paucity of measured data needed for the spin up, such as climatic data (average monthly temperature and precipitations), soil texture and pH and bulk density. The other parameters are considered internally of the model, once the type of ecosystems has been set (i.e. broadleaves rather than conifers). On the other side, the initialization of model and in particular soil compartment is not trivial. Actually the only way to overcome the measurements of the all soil C-pool is to fit the total modelled SOC to the total SOC measured at the site of interest in equilibrium or under a specific forest management as in Fig.5.

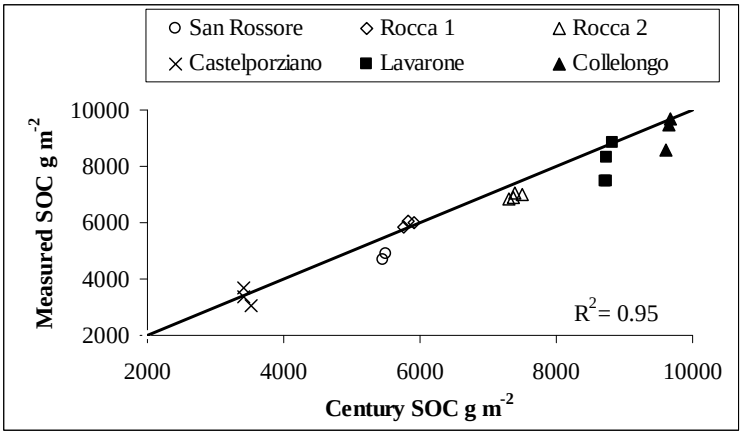

Fig. 5 Measured against modeled soil organic carbon stocks by Century model version 4.5 for several Alpine and Mediterranean forest sites. The line indicates the 1:1 relation. (Chiti et al. to be submitted).

After the initialization of the model in order to match the SOC modelled to the first available SOC measure, model evaluation has been performed using other data, directly measured or computed indirectly, such as net primary production (NPP) from Eddy-Covariance measurements, soil nitrogen stocks and soil respiration fluxes that have been compared to output model returned by Century. It was shown that once the model has been reasonably initialized and evaluated, compatibly with the available meteo and soil data, it is possible to have some confidence in the SOC changes detected by model simulations once different scenarios are performed (scenario A1F1: world markets-fossil fuel intensive, and scenario B2: local sustainability') as reported in Fig.6 as example.

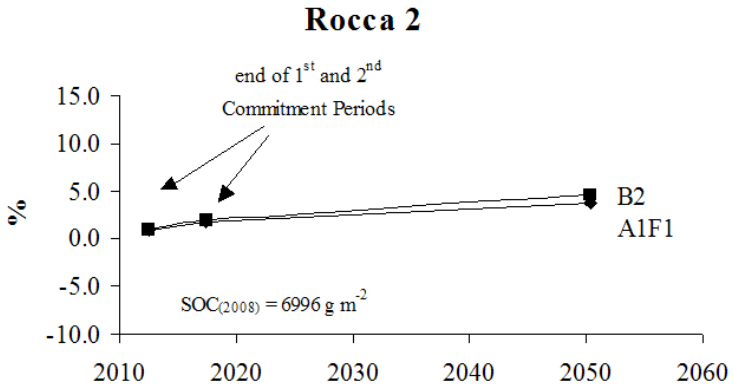

Fig. 6 Percentage variation in SOC amount compared to the reference year (2008) according to the climate change scenarios A1F1 and B2. Example reported for the site of Roccarespampani, Viterbo (Chiti et al. submitted).

Actually, as underlined by Chiti, the alternative solution on the short term is to perform a large soil sampling in order to detect the potential changes throughout the window of observation. If the number of samples is not in a sufficient number, the detected SOC change may be not statistically significant, making a more expensive and time-consuming field campaign necessary. In fact, as

reported in Fig. 5, the modelled SOC changes detected within the period 2008-2018 is quite narrow in this Mediterranean site (2%), requiring a large set of measured data to be statistically measured.
In conclusion, soil models such as Century, can be a complementary tool for estimating SOC dynamics on the short term in addition or in alternative to field measurements and on the long term in order to appreciate trend or estimate C stock changes. Presently N cycle is included in the Century soil module; the effects due to N deposition or increasing N availability are considered as affecting soil pH and NPP productivity, while no control on SOM decomposition is considered.

1.2.2 Soil models and the death soil paradigm: the role of soil microbes

In most of the soil models microbial community is considered as a further soil C-pool and not considered as a living system and/or explicitly expressed in degradation formulation (Fang et al.2005). The assumption at the basis of SOM decomposition supposing that decomposers are in equilibrium with substrates and degradation of C-pool has its equivalent in biomass growth without any intermediate step. However microorganisms are the key-driver of C and N dynamics and the feedbacks between the two cycles, thus when upscaling experimental evidences at process level to upper levels it is important to not loose information if microbes are not included explicitly.
Depending on the scale of observation, soil models exist taking account explicitly for a bacterial pool or even for an enzymatic pool where the degradation of soil organic matter is explicit function of this variables (Manzoni and Porporato 2009; Schimel and Weintraub 2003; Sinsabaugh et al.1994). The formulation permits to reproduce the priming effect and allows decomposition to be limited by both decomposers, substrates (Wurtzler and Reichstein 2008) and enzymatic pool as the first limiting step in degradation (Schimel and Weintraub 2003; Sinsabaugh and Moorhead 2000).
For example Sinsabaugh and co-authors underlined how activities constitute a new opportunity for a better understanding of microbial soil processes. They found as enzymatic cumulative rates were correlated with cumulative mass loss and how the instantaneous rates represented instantaneous measures of biochemical processes (Moorhead and Sinsabaugh 2000). Moreover an “economic model” was proposed where microbes maximize their productivity by optimizing the allocation of resources depending on the availability of nutrients (Sinsabaugh and Moorhead 1994, Sinsabaugh et al. 2002). Microorganisms allocate thus resources for the enzymatic expression depending on the need of C for biosynthesis processes and metabolism when concurrently constrained by the enzymatic production toward acquisition of nutrients (nitrogen and phosphorus). Although the basic idea is supported by the microbial need to obtain a specific ecological goal (i.e. growth rate maximization, fitness maximization), the model has been applied to litter and it is related to instantaneous enzymatic rates, making difficult the application to more general cases.

At the current state a lot of work has done to understand and model the C-N interaction on short temporal scale, i.e. soil incubation with residues, or on young substrates, i.e. fresh litter (see the review by Manzoni and Porporato 2009). In particular most of the soil schemes deal with condition of N limitation when decomposing C-rich substrates. Actually most of the soil microorganisms are heterotrophic, hence are firstly limited by C. However, when N-limitation occurs, a change of C-pathway has been underlined by several authors. In particular the following mechanisms have been proposed: a shift from anabolic to catabolic pathway, i.e. energy spilling (Schimel and Weintraub 2005; Neill and Gignoux 2006); shift from biomass synthesis toward emission of secondary products such as polysaccharides (Hadas et al.1998); decline of decomposition (Molina et al.1983; Hadas et al.1998). Contrarily experimental evidences showed a promotion of C-mineralization when N is added when microbes are growing on C-rich substrates such as wheat and straw (Green et al. 1995, Rescous et al.1995; Vestgarden 2001). However mineral N addition on litter exhibited experimentally different responses depending on nutrient status in soil rather than in litter (Knorr et al.2005; Vestgarden 2001). For example it has been shown that microbes are able to absorb N in organic form as small molecular weight compounds (Barraclough, 1997), hence this is likely to modulate the biological response to inorganic N amendment. Several soil N models differentiate thus if taking account for a MIT (Mineralization-immobilization) scheme, DIR (Direct organic N immobilization) scheme or both (Manzoni and Porporato 2009).

The effect of N surplus on litter and organic matter decomposition is still quite confused under a biochemical point of view. Presently ecosystem forest models deal with N promotion effect on net plant productivity (Century model, Mäkipää et al.1998; Salih et al.2005) but the potential for atmospheric N deposition to increase soil C storage via a reduction of SOM decomposition is a mechanism that is not considered or predicted in most models (Pepper et al. 2005), although the mechanisms may affect significantly the C budget on long temporal scales. Moreover C sequestration and stabilization is coupled to N sequestration in recalcitrant form and constitutes thus a process to be taken in to account.

To my knowledge few models exist trying to include the underlined mechanisms: the work proposed by Franklyn (2003) based on the continuous-quality decomposition theory and the dynamic models IBIS (Liu et al.2005) and SOMM model (Chertov and Komarov, 1997). However on one side there is the need to keep such models highly dynamic and manageable for predictive use, on the other hand it is important to maintain the biochemical information once upscaling the process in order to make useful the mathematical simplification.

Including the mechanisms that investigate the control of increased soil N status within forests, will help to better understand the response to N deposition and the feedbacks between vegetation and soil reducing uncertainties of net C-stock quantification.

Reference

Aber, J.D., Goodale, C.L., Ollinger, S.V., Smith, M.L., Magill, A.H., Martin, M.E., Hallet, R.A., Stoddard, J.L., 2003. Is nitrogen deposition altering the nitrogen status of northeastern forests? BioScience 53, 375–389.

Aber, J.D., Magill, A.H., 2004. Chronic nitrogen additions at the Harvard Forest (USA): the first 15 years of a nitrogen saturation experiment. Forest Ecology and Management 196, 1–5.

Aber, J., McDowell, W., Nadelhoffer, K., Magill, A., Berntson, G., Kamakea, M., McNulty, S., Currie, W., Rustad, L., Fernandez, I., 1998. Nitrogen Saturation in Temperate Forest Ecosystems, Hypoheses revisited. Bioscience 48, 921-934.

Ågreen, G.I., Bosatta, E., 2001. Combining theory and experiment to understand effects of inorganic nitrogen on litter decomposition. Oecologia 128, 94–98.

Allison, S.D., Vitousek, P.M., 2005. Responses of extracellular enzymes to simple and complex nutrient inputs. Soil Biology & Biochemistry 37, 937–944.

Allison, S.D., LeBauer, D.S., Ofrecio, M.R., Reyes, R., Ta, A., Tran, T., 2009. Low levels of nitrogen addition stimulate decomposition by boreal forest fungi. Soil Biology & Biochemistry 41, 293–302.

Alarcón-Gutiérrez, E., Couchaud, B., Augur, C., Calvert, V., Criquet, S., 2008. Effects of nitrogen availability on microbial activities, densities and functional diversities involved in the degradation of a Mediterranean evergreen oak litter (*Quercus ilex* L.). Soil Biology & Biochemistry 40, 1654–1661.

Baldrian, P., 2006. Fungal laccases occurrence and properties. FEMS Microbiol. Rev. 30, 215–242.

Barraclough, D., 1997. The direct or MIT route for nitrogen immobilization: a ^{15N} mirror image study with leucine and glycine. Soil Biology & Biochemistry 29, 101-108.

Berg, B., McClaugherty, C., 1987. Nitrogen release in relation to disappearance of lignin. Biogeochemistry 4, 219-224.

Berg, B., Matzner, E., 1997. Effect of N deposition on decomposition of plant litter and soil organic matter in forest ecosystems. Environ. Rev. 5, 1-25.

Berg, M.P., Kniese, J.P., Zoomer, R., Verhoef, H.A., 1998. Long-term decomposition of successive organic strata in a nitrogen saturated Scots pine forest soil. Forest Ecology and Management 107, 159–172.

Berg, B., Meentemeyer, V., 2002. Litter quality in a north European transect versus carbon storage potential. Plant and Soil 242, 83–92.

Bird, M.I., Santrùcková, H., Lloyd, J., Veenendaal, E.M., 2001. Global soil organic carbon pool. In: Schulze E-D; Harrison Sandy P; Heimann M; Holland EA; Lloyd J; Prentice I Colin; Schimel D (eds): Global biogeochemical cycles in the climate system. Academic Press, San Diego. pp. 185-197

Blagodatskaya, E., Kuzyakov, Y., 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. Biology and fertility of soil 45, 115-131.

Blagodatskiy, S.A., Larionova, A.A., Yevdokimov, I.V., 1993. Effect of mineral Nitrogen on the respiration Rate and Growth efficiency of soil microorganisms. Eurasian Soil Science 25, 85-95.

Blackwood, C.B., Waldrop, M.P., Zak, D.R., Sinsabaugh, R.L., 2007. Molecular analysis of fungal communities and laccase genes in decomposing litter reveals differences among forest types but no impact of nitrogen deposition. Environmental Microbiology 9, 1306-1316.

Bonan, G.B., 2008. Forests and Climate Change: Forcings, Feedbacks, and the Climate Benefits of Forests Science 320, 1444-1449.

Bowden, R.D., Davidson, E., Savage, K., Arabia, C., Steudler, P., 2004. Chronic nitrogen additions reduce total soil respiration and microbial respiration in temperate forest soils at the Harvard Forest. Forest Ecology and Management 196, 43–56.

Burton, S., 2003. Oxidizing enzymes as biocatalysts. Trends in Biotechnology 21, 543-549.

Butnor, J.R., Jhonsen, K.H., Oren, R., Katul, G.G., 2003. Reduction of forest floor respiration by fertilization on both carbon dioxide-enriched and reference 17-year-old loblolly pine stand. Global Change Biology 9, 849-861.

Caldwell, B.A., 2005. Enzyme activities as a component of soil biodiversity: A review. Pedobiologia 49, 637—644.

Carreiro, M.M., Sinsabaugh, R.L., Repert, D.A., Parkhurst, D.F., 2000. Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. Ecology 81, 2359–2365.

Cherif, M., Loreau, M., 2007. Stoichiometric constraints on resources use, competitive interactions, and elemental cycling in microbial decomposers. The American Naturalist 169, 709-724.

Chertov, O.G., Komarov,A.S., Nadporozhskaya, M.A., 2007. Analysis of the Dynamics of Plant Residue Mineralization and Humification in Soil. Eurasian Soil Science 40, 140-148.

Chertov, O.G., Komarov,A.S., 1997. SOMM: a model of soil organic matter dynamics. Ecological Modelling 94, 177-189.

Chiti, T., Papale, D., Smith, P., Dalmonech, D., Matteucci, G., Yeluripati, J., Rodeghiero, M., Valentini, R.. Predicting changes in soil organic carbon in mediterranean and alpine forests during the kyoto protocol commitment periods using the century model. Submitted to Soil Use and Managment.

Ciais, P., Tans, P.P., Trolier, M., White, J.W.C., Francey, R.J., 1995. A large northern hemisphere terrestrial CO_2 sink indicated by the 13C/12C ratio of atmospheric CO_2. Science 269, 1098–1102.

Claus, H., 2004. Laccases: structure, reactions, distribution. Micron 35, 93–96.

Coleman, K., Jenkinson, D.S., 1996. RothC-26.3 -- A model for the turnover of carbon in soil. In: Powlson, D.S., Smith, P., Smith, J.U. (Eds.), Evaluation of soil organic matter models using existing, long-term datasets. NATO ASI Series I, vol. 38. Springer, Berlin, pp. 237-246.

Conant, R.T., Smith, G.R., Paustian, K., 2003. Spatial Variability of Soil Carbon in Forested and Cultivated Sites: Implications for Change Detection. J. Environ. Qual. 32, 278–286.

Coûteaux, M., Bottner, P., Berg, B., 1995. Litter decomposition, climate and litter quality. Tree 10, 63-66.

Craine, J.M., Morrow, C., Fierer, N., 2007. Microbial nitrogen limitation increases decomposition. Ecology 88, 2105-2113.

Dale, V.H, Joyce, L.A, McNulty, S., Neilson, R.P., Ayres, M.P., Flannigan, M.D., Hanson, P.J., Irland, L.C., Lugo, A.E., Peterson, C.J., Simberloff, D., Swanson, F.G., Stocks, B.J., Wotton, B.M., 2001. Climate Change and Forest Disturbances. Bioscience 51, 723–734.

Davidson, E.A., Janssen, I.A., 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440, 165-173.

Davidson, E.A., Chorover, J., Dail, D.B., 2003. A mechanism of abiotic immobilization of nitrate in forest ecosystems: the ferrous wheel hypothesis. Global Change Biology 9, 228-236.

DeForest, J.L., Zak, D.R., Pregitzer, K.S., Burton, A.J., 2004. Atmospheric Nitrate Deposition, Microbial Community Composition, and Enzyme Activity in Northern Hardwood Forests. Soil Sci. Soc. Am. J. 68, 132–138.

Demoling, F., Nilsson, L.O., Bååth, E., 2008. Bacterial and fungal response to nitrogen fertilization in three coniferous forest soils. Soil Biology & Biochemistry 40, 370–379.

De Santo, A.V., Rutigliano, F.A., Berg, B., Fioretto, A., Puppi, G., Alfani, A., 2002. Fungal mycelium and decomposition of needle litter in three contrasting coniferous forests. Acta Oecol. 23, 247–259.

De Vries, W., Reinds, G.J., Gundersen, P., Sterba, H., 2006. The impact of nitrogen deposition on carbon sequestration in European forests and forest soils. Global Change Biology. 12, 1151-1173.

De Vries, W.,Solberg, S., Dobbertin, M., Sterba, H., Laubhann, D., van Oijen, M., Evans, C., Gundersen, P., Kros, J., Wamelink, G.W.W., Reinds, G.J., Sutton, M.A., 2009. The impact of nitrogen deposition on carbon sequestration by European forests and heathlands. Forest Ecology and Management 258, 1814–1823.

De Vries, W.,Solberg, S., Dobbertin, M., Sterba, H., Laubhann, D., van Oijen, M., Evans, C., Gundersen, P., Kros, J., Wamelink, G.W.W., Reinds, G.J., Sutton, M.A., 2009. The impact of nitrogen deposition on carbon sequestration by European forests and heathlands. Forest Ecology and Management 258, 1814–1823.

Dilly, O., Nannipieri, P., 2001. Response of ATP content, respiration rate and enzyme activities in an arable and a forest soil to nutrient additions. Biology and Fertility of Soils 34, 64–72.

Fang, C., Smith, P., Smith, J.U., Moncrieff, J.B., 2005. Incorporating microorganisms as decomposers into models to simulate soil organic matter decomposition. Geoderma 129, 139–146.

Fog, K., 1988. The effect of added nitrogen on the rate of decomposition of organic matter. Biological Reviews 63, 433–462.

Fontaine, S., Mariotti, A.,, Abbadie, L., 2003. The priming effect of organic matter: a question of microbial competition? Soil Biology & Biochemistry 35, 837–843.

Fontaine, S., Bardoux, G., Abbadie, L., Mariotti, A., 2004. Carbon input to soil may decrease soil carbon content. Ecology Letters 7, 314–320.

Fontaine, S., Barot, S. 2005. Size and functional diversity of microbe populations control plant persistence and long-term soil carbon accumulation. Ecology Letters 8, 1075–1087.

Franklin, O., Högberg, P., Ekblad, A., Göran, Ågren, G.I., 2003. Pine Forest Floor Carbon Accumulation in Response to N and PK Additions: Bomb ^{14}C Modelling and Respiration Studies. Ecosystems 2003 6, 644–658.

Frey, S.D., Knorr, M., Parrent, J.L., Simpson, R.T., 2004. Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. Forest Ecology and Management 196 (2004) 159–171.

Gallo, M, Amonette, R., Lauber, C., Sinsabaugh, R.L., Zak, D.R., 2004. Microbial Community Structure and Oxidative Enzyme Activity in Nitrogen-Amended North Temperate Forest Soils. Microbial Ecology 48, 218–229.

Galloway, J.N., Aber, J.D., Erisman, J. W., Seitzinger, S. P., Howarth, R. H., Cowling, E. B., Cosby, B.J., 2003. The nitrogen cascade. Bioscience 53, 341-356.

Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., Karl, D.M., Michaels, A.F., Porter, J.H. Porter, Townsend, A.R., Vörösmarty, C.J., 2004. Nitrogen cycles: past, present, and future. Biogeochemistry 70,153–226.

Green, C.J., Blackmer, A.M., Horton, R., 1995. Nitrogen Effects on Conservation of Carbon during Corn residue Decomposition in Soil. Soil Sci. Soc. Am. 59, 453-459.

Gruber, N., Galloway, J.N., 2008. An Earth-system perspective of the global nitrogen cycle. Nature 17, 293-296.

Hadas, A., Parkin, T.B., Stahl, P.D., 1998. Reduced CO_2 release from decomposing wheat straw under N-limiting conditions: simulation of carbon turnover. European Journal of Soil Science 49, 487-494.

Hagedorn, F., Spinnler, D., Siegwolf, R., 2003. Increased N deposition retards mineralization of old soil organic matter. Soil Biology & Biochemistry 35, 1683–1692.

Hart, S.C., Nason, G.E., Myrold, D.D., Perry, D.A., 1994. Dynamics of gross nitrogen transformation in an old-growth forest: the carbon connection. Ecology 75, 880-891.

Henriksen, T.M., Breland, T.A., 1999. Nitrogen availability effects on carbon mineralization, fungal and bacterial growth, and enzyme activities during decomposition of wheat straw in soil. Soil Biology & Biochemistry 31, 1121-1134.

Hyvönen, R., Persson, T., Andersson, S., Olsson, B., Ågren, G.I., Linder, S., 2008. Impact of long-term nitrogen addition on carbon stocks in tree and soils in northern Europe. Biogeochemistry 89, 121-137.

Hofmockel, K.S., Zak, D.R:, Blackwood, C.B., 2007. Does Atmospheric NO_3 Deposition Alter the Abundance and Activity of Ligninolytic Fungi in Forest Soils? Ecosystems 10, 1278–1286.

Högberg, M.N., Högberg, P., Myrold, D.M., 2007. Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three? Oecologia 150, 590–601.

Holland J.N., Cheng W., Crossley D.A., 1996. Herbivore-induced changes in plant carbon allocation: assessment of below-ground C fluxes using carbon-14. Oecologia, 107 87–94.

Hungate, B.A., Dukes, J.S., Shaw, M.R., Luo, Y., Field, C.B., 2003. Nitrogen and Climate Change. Nature 302, 1512.

Jacobson, M.C., Charlson , R.J., Rodhe, H., Orian,s G.H., 2000. Earth System Science. Academic Press.

Janssens, I.A., Sebastiaan, L., 2009. Carbon cycle: Nitrogen's carbon bonus. Nature Geoscience 2, 309-370.

Kirschbaum M.U.F., Paul K.I., 2002. Modelling C and N dynamics in forest soils with a modified version of the Century model. Soil Biology & Biochemistry 34, 341-354.

Knorr, M., Frey, S.D., Curtis, P.S., 2005. Nitrogen additions and litter decomposition: a meta-analysis. Ecology 86, 3252-3257.

Lagomarsino, A., Knapp, B.A., Moscatelli, M.C.,, De Angelis, P., Grego, S., Insam, H., 2007. Structural and Functional Diversity of Soil Microbes is Affected by Elevated [CO2] and N Addition in a Poplar Plantation. J Soils Sediments 7, 399–405.

Liu, J., Price, D.T., Chen, J.M., 2005. Nitrogen controls on ecosystem carbon sequestration: a model implementation and application to Saskatchewan, Canada. Ecological Modelling 186, 178–195.

Magill A.H., Aber, J.D., Hendricks, J.J., Bowden, R.D., Melillo, J.M., Steudler, P.A., 1997. Biogeochemical response of forest ecosystems to simulated chronic nitrogen deposition. Ecological application 7, 402-415.

Magill, A.H., Aber, J.D., 1998. Long-term effects of experimental nitrogen additions on foliar litter decay and humus formation in forest ecosystems. Plant and Soil 203, 301–311.

Magill, A., Aber, J.D., 2000. Dissolved organic carbon and nitrogen relationships in forest litter as affected by nitrogen deposition. Soil Biology & Biochemistry 32, 603-613.

Magnani, F., Mencuccini, M., Borghetti, M., Berbigier, P., Berninger, F., Delzon, S., Grelle, A., Hari, P., Jarvis, P.G., Kolari, P., Kowalski, A.S., Lankreijer, H.L., Law, B.E., Lindroth, A., Loustau, D., Manca, G., John, B., Moncrieff, J.B., Rayment, M., Tedeschi, V., Valentini, R., Grace, J., 2007. The human footprint in the carbon cycle of temperate and boreal forests. Nature 447, 848-852.

Mäkipää, R., 1995. Effect of nitrogen input on carbon accumulation of boreal forest soils and ground vegetation. Forest Ecology and Management 79, 217-226.

Manzoni, S., Jackson, R.B., Trofymow, J.A., Porporato, A., 2008. The Global Stoichiometry of Litter Nitrogen Mineralization Science 321, 684-686.

Manzoni, S., Porporato, A., 2009. Soil carbon and nitrogen mineralization: Theory and models across scales. Soil Biology & Biochemistry xxx, 1–25.

Melillo, J.M., Aber, J.D., Muratore, J.F., 1982. Nitrogen and Lignin Control of Hardwood Leaf Litter Decomposition Dynamics. Ecology 63, 621-626.

Melillo, J.M., McGuire, A.D., Kicklighter, D.W., Moore,III,B., Vorosmarty, C.J., Schloss, A.L.,1993. Global climate change and terrestrial net primary production. Nature 363, 234-240.

Melillo, J.M., Steudler, P.A., Aber, J.D., Newkirk, K., Lux, H., Bowles, F.P., Catricala, C., Magill, A., Ahrens, T., Morrisseau, S., 2002. Soil warming and carbon-cycle feedbacks to the climate systems. Science 298, 2173–2176.

Mellert et al.2004

Michel, K., Matzner, E., 2003. Response of enzyme activities to nitrogen addition in forest floors of different C-to-N ratios. Biol. Fertil. Soils 38, 102–109.

Micks, P., Aber, J.D., Boone, R.D., Davidson, E.A., 2004. Short-term soil respiration and nitrogen immobilization response to nitrogen applications in control and nitrogen-enriched temperate forests. Forest Ecology and Management 196, 57–70.

Miller, M., Palojärvi, A., Ranger, A., Reeslev, M., Kjøller, A., 1998. The Use of Fluorogenic Substrates To Measure Fungal Presence and Activity in Soil. Applied and environmental microbiology 64, 613–617.

Molina, J.A.E., Clapp, C.E., Shaffer, M.J., Chichester, F.W., Larson, W.E., 1983. NCSOIL, a model of nitrogen and carbon transformation in soil: description, calibration and behaviour. Soil Sci. Soc. Am. J. 47, 85-91.

Moorhead, D.L., Sinsabaugh, R.L., 2000. Simulated patterns of litter decay predict patterns of extracellular enzyme activities. Applied Soil Ecology 14, 71-79.

Moran, K.K., Six, J., Horwath, W.R., van Kessel, C., 2005. Role of Mineral-Nitrogen in Residue Decomposition and Stable Soil Organic Matter Formation. Soil Sci. Soc. Am. J. 69, 1730–1736.

Nabuurs, G.J., O. Masera, K. Andrasko, P. Benitez-Ponce, R. Boer, M. Dutschke, E. Elsiddig, J. Ford-Robertson, P. Frumhoff, T. Karjalainen, O. Krankina, W.A. Kurz, M. Matsumoto, W. Oyhantcabal, N.H. Ravindranath, M.J. Sanz Sanchez, X. Zhang, 2007: Forestry. In Climate Change 2007: Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [B. Metz, O.R. Davidson, P.R. Bosch, R. Dave, L.A. Meyer (eds)], Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Nadelhoffer, K., et al., 2004. Decadal-scale fates of N-15 tracers added to oak and pine stands under ambient and elevated N inputs at the Harvard Forest (USA). Forest Ecology and Management 196, 89–107.

Nadelhoffer, K. J. *et al*. 1999. Nitrogen deposition makes a minor contribution to carbon sequestration in temperate forests. Nature 398, 145–148.

Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G., Renella, G., 2003. Microbial diversity and soil functions. European Journal of Soil Science 54, 655–670.

Neff, J.C., Townsend, A.R., Gleixner, G., Lehman, S., Turnbull, J., Bowman, W.D., 2002. Variable effects of nitrogen additions on the stability and turnover of soil carbon. Nature 419, 915–917.

Neill, C., Gignoux, J., 2006. Soil organic matter decomposition driven by microbial growth: A simple model for a complex network of interactions. Soil Biology & Biochemistry 38, 803–811.

Nohrstedt, H.Ő., Arnebrant, K., Bååth, E., Söderström, B., 1988. Changes in carbon content, respiration rate, ATP content, and microbial biomass in nitrogen-fertilize pine forest soils in Sweden. Ca. J. For. Res. 19, 323-328.

Ohtonen, R., and Markkola, A.M. 1991. Biological activity and amount of FDA mycelium in mor humus of Scots pine stands (*Pinus sylvestris* L.) in relations to soil properties and degree of pollution. Biogeochemistry 13, 1–26.

Olander, L.P., Vitousek, P.M., 2000. Regulation of soil phosphatase and chitinase activity by N and P availability. Biogeochemistry 49, 175–190.

Olsson, P., Linderw, S., Slerz, R., Högberg, P., 2005. Fertilization of boreal forest reduces both autotrophic and heterotrophic soil respiration. Global Change Biology 11, 1745–1753.

Oren, R., Ellsworth, D.S., Johnsen, K.H., Phillips, N., Ewers, B.E., Maier, C., Schafer, K.V.R., McCarthy, H., Hendrey, G., McNulty, S.G., Katul, G.G., 2001. Soil fertility limits carbon sequestration by forest ecosystems in a CO2-enriched atmosphere. Nature 411, 469-472.

Parton, W.J., Schimel, D.S., Cole, C.V. & Ojima, D.S. 1987. Analysis of factors controlling soil organic matter levels in Great Plains grasslands. Soil Science Society of America Journal 51, 1173-1179.

Parton, W.J., Stewart, J.W.B., Schimel, D.S., 1988. Dynamics of C,N,P and S in grassland soils: a model. Biogeochemistry 5, 109-131.

Parton, W.J., Scurlock, J.M.O., Ojima, D.S., Gilmanov, T.G., Scoles, T.G., Schimel, D.S., Kirchner, T., Menaut, J.C., Seastedt, T., Garcia Moya, E., Kamnalrut Apinan, Kinyamario, J.I., 1993. Observations and modeling of biomass and soil organic matter dynamics for the grassland biome worldwide. Global Biogeochemical Cycles 7, 785–809.

Pepper, D.A., Del Grosso, R.E., McMurtrie, R.E., Parton, W.J., 2005. Simulated carbon sink response of shortgrass steppe, tallgrass prairie and forest ecosystems to rising [CO_2], temperature and nitrogen input. Global Biogeochemical Cycles, 19, 1-20.

Persson, T., Karlsson, P.S., Seyferth, U., Sjöberg, R.M., Rudebeck, A.. in *Carbon and nitrogen cycling in European forest ecosystems* (ed. Schulze, E. D.) 257–275 (Ecological Studies 142, Springer-Verlag, Berlin, 2000).

Pregitzer, K.S., Burton, A.J., Zak, D.R., Talhelm, A.F., 2008. Simulated chronic nitrogen deposition increases carbon storage in Northern Temperate forests. Global Change Biology 14, 142–153.

Prentice, I.C., Farquhar, G.D., Fasham, M.J.R., Goulden, M.L., Heimann, M., Jaramillo, V.J., Kheshgi, H.S., Le Que´re´, C., Scholes, R.J., Wallace, D.W.R., 2001. Pages 183–237 *in* J. T. Houghton and D. Yihui, editors. Climate change 2001: the scientific basis. The Intergovernmental Panel on Climate Change (IPCC) third assessment report. Cambridge University Press, Cambridge, UK.

Prescott,C.E., 1995. Does nitrogen availability control rates of litter decomposition in forests? Plant and soil 168-169, 83-88.

Rayner, P. J., Enting, I.G., Francey, R.J., Langenfelds, R., 1999. Reconstructing the recent carbon cycle from atmospheric CO_2, d13C, and O_2/N_2 observations. Tellus 51B:213–232.

Reay, D.S., Dentener, F., Smith, P., Grace, J., Feeley, R.A., 2008. Global nitrogen deposition and carbon sink. Nature Geoscience 1, 430-437.

Recous, S., Dobin, D., Darwin, D., Mary, B., 1995. Soil inorganic N availability: effect on maize residue decomposition. Soil Biology & Biochemistry 27, 1529-1538.

Reich, P.B., Hungate, B.A., Luo, Y., 2006. Carbon-Nitrogen Interactions in Terrestrial Ecosystems in Response to Rising Atmospheric Carbon Dioxide. Annu. Rev. Ecol. Evol. Syst. 37, 611-636.

Ryan, M.G:, Law, B.E., 2005. Interpreting, measuring, and modeling soil respiration Biogeochemistry 73, 3–27.

Romanyà, J., Cortina, J., Falloon, P., Coleman, K., Smith, P., 2000. Modeling changes in soil organic matter after planting fastgrowing Pinus radiata on Mediterranean agricultural soils. Eur. J. Soil Sci. 51, 627– 641.

Rustad, L.E., Campbell, J.L., Marion, G.M., Norby, R.J., Mitchell, M.J., Hartley, A.E., Cornelissen, J.H.C., Gurevitch, J., 2001. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental eco system warming. Oecologia 126, 543–562.

Saiya-Cork, K.R., Sinsabaugh, R.L., Zak, D.R., 2002. The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil ,2002. Soil Biology & Biochemistry 34, 1309–1315.

Salih, N.,Ågren, G.I., Hallbäcken, L., 2005. Modeling reponse of N addition on C and N allocation in Scandinavian Norway spruce stands. Ecosystems 8, 373-381.

Shibu, M.E., Leffelaar, P.A., Van Keulen, H., Aggarwal, P.K., 2006. Quantitative description of soil organic matter dynamics—A review of approaches with reference to rice-based cropping systems. Geoderma 137, 1–18.

Schimel D.S., Braswell Jr E.A., Holland R., Mc-Keown D.S et al., 1994. Climatic, edaphic and biotic controls over storage and turnover of carbon in soils. Global Biochemical Cycle 8, 279-293.

Schimel, J.P., Weintraub, M.N., 2003. The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biology & Biochemistry 35, 549–563.

Schimel, J.P., Bennett, J., 2004. Nitrogen mineralization: challenges of a changing paradigm Ecology, 85, 591–602.

Schulten, H.R., Schnitzer, M., 1998. The chemistry of soil organic nitrogen: a review. Biology and fertility of soils 26, 1-15.

Sinsabaugh, R.L., Moorhead, D.L., 1994. Resources allocation to extracellular enzyme production: a model for nitrogen and phosphorus control of litter decomposition. Soil Biology & Biochemistry 26, 1305-1311.

Sinsabaugh, R.L., Carreiro, M.M., Repert, D.A., 2002. Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. Biogeochemistry 60, 1–24.

Sinsabaugh, R.L., Zak, D.R., Gallo, M.E., Lauber, C., Amonette, R., 2004. Nitrogen deposition and dissolved organic carbon production in northern temperate forests. Soil Biology & Biochemistry 36, 1509–1515.

Sinsabaugh, R.L., Gallo, M.E., Lauber, C., Waldrop, M.P., Zak, D.R., 2005. Extracellular enzyme activities and soil organic matter dynamics for northern hardwood forests receiving simulated nitrogen deposition. Biogeochemistry, 75: 201–215.

Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosta, A.L., Cusack, D., Frey, S., Gallo, M.E., Gartner, T.B., Hobbie, S.E., Holland, K., Keeler, B.L., Powers, J.S., Stursova, M., Takacs-Vesbach, C., Waldrop, M.P., Wallenstein, M.D., Zak, D.R., Zeglin, L.H., 2008. Stoichiometry of soil enzyme activity at global scale. Ecology letters, 11: 1252–1264.

Sjöberg, G., Nilsson, S.I., Persson, T., Karlsson, P., 2004. Degradation of hemicellulose, cellulose and lignin decomposing spruce needle litter in relation to N. Soil Biology & Biochemistry 36, 1761-1768.

Sjöberg, R.M., Persson, T., 1998. Turnover of carbon and nitrogen in coniferous forest soils of different N-status and under different $^{15}NH_4$-N application rate. Environmental Pollution 102, 385-393.

Södeström, B., Bååth, E., Lundgren, B., 1983. Decrease in soil microbial activity and biomasses owing to nitrogen amendments. Can. J. Microbiol. 29, 1500-1506.

Sutton, M.A., Simpson, D., Levy, P.E., Smith, R.I., Reis, S., Van Oijen, M., De Vries, W., 2008. Uncertainties in the relationship between atmospheric nitrogen deposition and forest carbon sequestration Global Change Biology 14, 2057–2063.

Swanston, C., Homann, P.S., Caldwell, B.A., Myrold, D.D., Ganio, L., Sollins, P. 2004. Long-term effects of elevated nitrogen on forest soil organic matter stability. Biogeochemistry **70,** 227–250.

Thuiller, W., Albert, C., Arau´ jo, M.B., Berry, P.M., Cabeza, M., Guisan, A., Hickler, T., Midgley, G.F., Paterson, J., Schurr, F.M., Sykes, M.T., Zimmermann, N.E., 2008. Predicting global change impacts on plant species' distributions: Future challenges. Perspectives in Plant Ecology, Evolution and Systematics 9, 137-152.

Tirukkumaran, C.M., Perkinson, D., 2000. Microbial respiration, biomass, metabolic quotient and litter decomposition in a lodgepole pine forest floor amended with nitrogen and phosphorous fertilizers. Soil Biology & Biochemistry 32, 59-66.

Tirukkumaran, C.M., Perkinson, D., 2002. Microbial activity, nutrient dynamics and litter decomposition in a Canadian Rocky Mountain pine forest as affected by N and P fertilizers. Forest Ecology and Management 159, 187–201.

Townsend, A. R., Braswell, B. H., Holland, E. A., Penner, J. E., 1996. Spatial and temporal patterns in terrestrial carbon storage due to deposition of fossil fuel nitrogen. Ecological Application 6, 806–814.

Trumbore S., 2006. Carbon respired by terrestrial ecosystems - recent progress and challenges. Glob Change Biol 12, 141-153.

Tuncer, M., Kuru, A., Isikli, M., Sahin, N., Celenk, F.G., 2004. Optimization of extracellular endoxylanase, endoglucanase and peroxidase production by Streptomyces sp. F2621 isolated in Turkey. Journal of Applied Microbiology 97, 783–791.

Valentini, R., Matteucci, G., Dolman, A.J., Schulze E.D., 2003. The role of canopy flux measurements in global C-cycle research, in: Fluxes of Carbon, Water and Energy of European Forests, Valentini R.(ed.), Springer Verlag, 255-266.

Vestgarden, L.S., 2001. Carbon and nitrogen turnover in the early stage of Scots pine (*Pinus sylvestris L.*) needle litter decomposition: effects of internal and external nitrogen. Soil biology & biochemistry 33, 465-474.

Vitousek, P.M., et al., 1998: Within-system element cycles, input-output budgets, and nutrient limitations. In: *Successes, Limitations, and Frontiers in Ecosystem Science* [Pace, M., and P. Groffman (eds.)]. Springer-Verlag, New York, pp. 432–451.

Waldrop, M.P., Zak, D.R., Sinsabaugh, R.L., 2004. Microbial community response to nitrogen deposition in northern forest ecosystems. Soil Biology & Biochemistry 36, 1443–1451.

Waldrop, M.P., Zack, D.R., 2006. Response of Oxidative Enzyme Activities to Nitrogen Deposition Affects Soil Concentrations of Dissolved Organic Carbon. Ecosystems 9, 921–933.

Wutzler, T., Reichstein, M., 2008. Colimitation of decomposition by substrate and decomposers – a comparison of model formulations. Biogeosciences Discuss 5, 163–190.

Zak, D.R:, Pregitzer, K.S., Holmes, W.E., Burton, A.J., Zogg, G.P., 2004. Anthropogenic N deposition and the fate of $^{15}NO^{-}_{3}$in a northern hardwood ecosystem. Biogeochemistry 69, 143-157.

1.3 Objectives of the study

The main focus of the present work was to gain more understanding within the context of soil C-cycle under different soil N status and the feedbacks between the two, focussing on the role of microorganisms and their capability to respond and adapt to changed environmental conditions.
In the first section a review of the soil fertilization experiments performed in both field and laboratory has been presented. All the experiments where enzymatic activities were monitored were included in order to summarize the main findings and to understand the gaps to be filled in the topic of C and N interaction concerning experiments and conceptualization of the problem proposing for the first time a scheme trying to include all the experimental evidences.
The main issues were thus addressed toward the microbial role and their ecological functions by medium of an incubation experiments performed in laboratory with addition of mineral N that is characterized by the novelty of the set up proposed and the type of ecosystem investigated. Moreover the present study was addressed *via* mathematical tools, in particular developing a soil model of the decomposition of litter and SOM accumulation solving numerically the ordinary differential equations describing the biochemical soil processes. In particular I started from the soil basic C-scheme of Century and including the new N cycle, modulating the response of C dynamics under the presence of organic and mineral N not considered previously in current soil models, in particular on the long term. The overall goal was thus to investigate the microbial response and their control on C and N dynamics and to provide a formulation for a dynamical model describing the accumulation of C under increasing N availability at the plot scale. In the last phase of the work , the soil C and N dynamics were investigated under an interesting and fashinating point of view, the microbial adaptive behaviour. I applied thus some well known concepts in animal behaviour area, to the contest of soil decomposition processes and C sequestration.
The work is subdivided thus into different topics, according to the specific objectives and various methodological approaches used to attain them.

1.3.1 Study approach and summary of main findings

Chapter 2. How does the organic matter decomposition respond to mineral N addition in a Mediterranean soil? Do similarities exist across sites regardless of direct climatic control?

Among the several N fertilization experiments, a lot of attention has moved particularly toward temperate forests of North Europe and North America while little is know about N availability impact on Mediterranean forest soils with regard to C-dynamics and microbial activity. In particular

the focus is the role of increasing mineral N availability due to several effects such as N deposition, rather than increasing SOM mineralization due to increasing temperature. For instance, Mediterranean area is indicated as a highly sensitive to climate change, to land use and to any kind of external disturbance. Although the major issues of these sensitive areas are of hydrological nature, on the other side it is important to investigate the dynamics following the N cycle being crucial to soil C dynamics and being a limiting factor to plant productivity. Moreover, due to the low C-pool size, it is critical to investigate any direct and indirect mechanism that may potentially alter this stock.

In the last years more attention has given mainly to Mediterranean forest litters decomposition due to abiotic constrains (i.e. temperature and humidity); the experiment that has been carried out is thus the first work of N fertilization performed on an organic soil of a Mediterranean forest. The aim to carry out the soil experiment emerges from the need to investigate and verify if a correlation exists between the biochemical variables and the real mineral N availability and which type of response can exhibit a soil originated from a Mediterranean climate type and an ecosystem completely different from the previous ones studied. Soil laboratory incubation allows studying the microbial dynamics under N addition with a major control on the biochemical response and in a narrower temporal window compared to field experiments. The incubation was conducted fertilizing soil with different amount of inorganic N added in small aliquots among incubation time, in order to simulate a slow increase of mineral N in the soil solution and to study the response of the system. I investigated thus if a correlation with inorganic N present in soil solution and with incubation time exists. The experiment was performed setting a complete and integrates monitoring activity including the measurements of C-mineralization among the analyses of a suitable set of enzymes and substrates utilization pattern. Although the experiment was carried out under optimal condition of temperature, moisture and nutrient (P limitation was avoided) several results have been highlighted.

The soil showed in the first period a response similar to the response observed in a site covered by an oak-type vegetation in north America, supporting the hypothesis that on old organic matter originated by low-quality litter the recalcitrance may control the microbial response to N addition with a minor direct control of climatic factors. The results suggested how in a Mediterranean soil, the surplus of mineral N may have an important role among other abiotic factors for this reason that can not be excluded in evaluating soil C-balance.

Besides, the experimental data would suggest that under labile C availability in combination of mineral N, a promotion of C stock could occur. When there is not a continuous input of labile C a stress condition may occur on long temporal scale, microbial community is negatively affected and induces a C loss from the system. Hence, there is a confirmation that that acidification process due

to N addition can not be adduced as the only contribution to suppression of microbial activity but changes in microbial functionality may contribute significantly as well.

Chapter 3. A new formulation of microbial decomposition control under increasing N availability

The approach proposed to investigate the role of soil N status on soil C dynamics is the development of a simple model that starts from litter decomposition and follows C-transformation from fresh compounds to recalcitrant-C and accumulation of organic matter. The model study is mainly devoted to mineral N control on long temporal scale and how N availability controls SOM decomposition and thus C cycle with particular attention to the biochemical behaviour of microorganisms.

Since once organic matter interacts with mineral matrix, several physical and chemical mechanisms of protection and stabilization may play a major role over other abiotic effect, I focus the attention on humus formation and accumulation. The focus of the work was addressed toward C and N interactions under increasing N availability behind climatic factors. The basic hypothesis is that climatic conditions drive mainly quantity and quality of litter among N availability, promoting SOM mineralization, while the feedback of mineral N on humus decomposition operates mainly due to the coupling with recalcitrance.

The goal was to propose a model that started from the Century C-scheme and revisited the N cycle focussing on the feedbacks between the two cycles with attention to organic and inorganic N. The merit of the work was to the modelling of a process presently not yet completely included in dynamic models. Indeed the effort was to find a trade off between the complexity of the biochemical processes and the modelling approach than could maintain the most judicious mathematical simplification in order to be manageable and easy to be interfaced with the models currently present in literature. The formulation expresses the biological response to increasing N availability as function of the microbial N need and mineral N availability in the soil. The model was calibrated and validated using a litterbag study conducted at the experimental forest site at Harward University. A sensitive and qualitative study followed in order to evaluate the capability to sequester C under different internal and external N levels. The final purpose of this work is to find a trade off between the ecological issue at biological level and the need for keeping soil model manageable using semi-empirical relationships without considering soil simply as a black box, by-passing the dead-soil paradigm and without loosing the mechanism at process level.

The present model is able to reproduce the main experimental evidences and the potential soil accumulation starting from litter degradation depending on availability of external (N deposition,

increased SOM mineralization realising mineral N) and internal (N litter concentration) N. Actually the formulation of N inhibition is function of litter characteristics allowing the application to different ecosystems characterized by litter of different quality, in particular lignin and N concentration. Although a qualitative study was performed without taking account explicitly of climatic factors, the results obtained when mimicking an increase of litter N are in line with data reported in literature by other authors.

The model fails in reproducing the humus build-up observed in boreal forests. The reasons can be found in the missing vertical development of the model that does not allow reproducing the accumulation of several layer of organic matter characterized by different amount of total N.

However, with a suitable model optimization, the showed modelling capability makes the model robust against applicability for predictive use rather than coupled to plant sub-models. The use of external available or easy measurable variables among the paucity of parameters to be optimized, allow the model to be highly manageable. Besides, the use of data concerning the litter quality in the formulation includes implicitly the microbial activity and in particular the enzymatic production. Actually litter chemistry has been always constituted the best way to upscale the mechanism at process level to ecosystem scale. Moreover the control on decomposition is explicit function of soil mineral N that is usually computed automatically in the current models. Hence, keeping the same mathematical formulation, the proposed work makes us confident in the robustness of model to investigate dynamically the soil C and N interactions.

Chapter 4. Substrate preference of microbial community in soil decomposition models: a new perspective.

In the last section of the present work I modified some known soil models from literature in order to take account of adaptive behaviour of microorganisms when feeding on more substrates. Markedly the work focuses on the pattern of substrate preference when the substrates of interests are litter and native soil. However, without loss of generality, the mathematical formulation and the obtained results hold for any type of substrates. In fact the proposed models are based on ecological concepts with regards to the animal behaviour and microeconomics, hence the allocation of resources in order to have a specific return from the investments. Despite the formulations are really simple, the merit of the work was to try to explain some experimental evidences (i.e. priming on long temporal scales) with regards to both C and N, with few parameters and variables that can be easily measured. Besides, the optimization model, although in a first attempt, offers a new tools for investigating the soil response to external disturbance analogously to the recent plant models utilizing optimization rules.

1.3.2 Conclusions

The evaluation of mineral N pattern within forest ecosystems constitutes an important and crucial issue for determining the potential for C sequestration and its feedbacks to climate change. In particular the review of the different studies reported in literature, underlined how the plant and soil response to increasing N availability (artificial fertilization rather than N deposition) depends itself on soil N status. In particular the capability to retain N in mineral form rather than in recalcitrant form affects in turn C sequestration in humus and modulates N availability for aboveground vegetation.

The work focused in particular on the role of mineral N on microbial activity and thus on soil response. From literature overview it erased that the mechanisms behind the microbial response are several and a common point it still far from a coherent frame. Indeed the macroscopic experimental evidence resulted in a suppression of biological activity, thus lower respiration and a trend toward a C-sink. However different hypothesis have been supported in literature, including mainly biochemical effects on soil microorganisms, but although a general scheme may be achieved suggesting explanations of energetic and efficiency nature, the real mechanisms and the temporal scale of occurrence are still far from a complete understanding.

Thereinafter in this study I went in to deep of the response of soil C dynamics to increasing N availability *via* experimental data analyses and modelling, focussing on the interaction between the two cycles and their feedbacks. A particular attention was dedicated to the role of microbial community as the main determinant of the soil response.

The novelty of the experimental set up underlined interesting critical points concerning the mineral N control on soil processes. The obtained results suggested the importance of C-labile presence in the determination of C pathways under N deposition. In particular it seemed that N may promote C sequestration until the C labile is finished and the toxic amount of mineral N causes a microbial stress with loss of C from the systems. In particular it is important to mention that the experiment was conducted on a soil characterized by a low C/N ratio while other experiments were conducted mostly in N-limited forests of soil with larger C/N ratio. The occurrence of a critical stress point could be hence justifiable due to the reaching of a condition of saturation, although it is difficult to extrapolate to a general scheme. Although some of the aspects underlined in the experiment are known in literature, the work has the merit to be able to individuate different patterns within the same windows of observation. Besides, the results obtained in the first period of incubation make us confident in suggesting how increasing mineral N may have a similar response on recalcitrant C

decomposition with a minor direct control of the climatic conditions of the site of origin, a result that was not obvious.

The proposed soil model and its mathematical formulation represent a trade off between a complex formulation with increasing number of parameters and the need to include the most important biochemical information in the current dynamic ecosystem models.

The developed soil model is able to reproduce the main experimental evidences and the potential soil accumulation starting from litter degradation depending on availability of mineral N provided externally *via* N deposition rather than increased SOM mineralization and internal due to increasing foliar N. The model returns that an accumulation of C always occurs until the surplus of N is not reduced by plants or other type of loss rather than a decline of external input since the inhibitory mechanism is not permanent but stops when mineral N soil decreases again. The qualitative study included hence all the phenomena that lead to an increase of mineral N in to the soil.

The formulation proposed allowed to speculate about some critical issue. Indeed if a raised temperature due to climate change leads to a promotion of SOM mineralization and in turn to a higher mineral N availability, the C loss due to increased temperature may be counteract by the suppressive effect of mineral N if is retained within soil. Particularly when N diffusion occurs between soil layers characterized by C-pools of different quality.

Although the model was not optimized on a large set of data and the soil scheme is mono-dimensional, the model evaluation and the qualitative study results make confident in the working hypothesis behind the model and in the potential use of this soil module coupled to a vegetation model. Indeed it is important to keep in mind firstly the potential for plant competition for available N, secondly the potential benefit of N addition on promotion on C stock in the forests could be offset by the increase of N_2O emissions having a higher warming potential than CO_2.

The qualitative analyses in the last phase of the present work higlighted the important role of microorganisms in the mediation of soil response to changed environmental condition and in particular the quality and availability of C and N resources. In particular including the adaptive behaviour and thus how microbial choice towards different substrates changes following a specific ecological goal, appears a promising tool for the future. This would allow to generalize the microbial behaviour and to organize all the experimental data in a coherent scheme able to detect the magnitude and the direction of C-sequestration.

2. N FERTILIZATION EXPERIMENT ON A MEDITERRANEAN FOREST SOIL

Keywords: *N availability, microbial community, C-turnover, enzymes, CLPP, functional diversity*

2.1 Introduction

In temperate regions soil inorganic nitrogen (N) availability can be altered by several factors. It increased in response to increased mineralization due to rising temperature (Rustad et al., 2001; Melillo et al., 2002) or changed hydrological regime and increased N-external input through N depositions and fertilizers. Changes in these factors are mainly due to the indirect effects of atmospheric emission of anthropogenic greenhouse gases and the production of reactive N (Galloway et al., 2003).

In undisturbed forest ecosystems, N inputs are mainly constituted by N-deposition, although a contribution may come from biological N-fixation. Increased of organic matter decomposition (through its concomitant release of N) provides an almost continuous input to the mineral soil pool. However the N depositions appears to be the most important contribution. Indeed, in European forests wet and dry N depositions are reported to range between 10 and 75 kg ha^{-1} y^{-1} (Gundersen et al., 1998; Macdonald et al., 2002) with an increasing trend, suggesting a high potential for rising inorganic N (N_{in}) availability within these ecosystems.

In forests, soil inorganic N availability controls, directly and indirectly, processes such as litter and soil organic matter (SOM) decomposition, and plant productivity (Berg and Matzner, 1997; Magnani et al., 2007). Hence increasing N inputs can alter the carbon (C) balance in these natural ecosystems, thus affecting the potential of forests to mitigate the increasing CO_2 concentration in the atmosphere (Hungate et al., 2003).. At present the magnitude and the direction of soil response to increasing N_{in} are still under debate. In fact, the soil reacts to an increase of N_{in} according to several factors such as the type of ecosystem, litter quality and microbial community (Waldrop et al., 2004; Sinsabaugh et al., 2005, 2008; Allison et al., 2009), the rate of fertilization, and N demand (Knorr et al., 2005). Although it is difficult to find a clear and predictable pattern, the review by Reay et al.(2008) suggested a general trend of forest soils becoming C-sinks in response to an increase of N_{in} availability. Soil response is mediated markedly by microorganisms, due to their involvement in organic matter dynamics (Nannipieri et al., 2003, Caldwell, 2005). However, there is mixed supporting evidence for the currently proposed microbial mechanisms underlying the biochemical response to N_{in} increase

In the last 20 years soil incubation experiments with N fertilization have been performed on organic/mineral soils and litter (Berg and Matzner, 1997; Tirukkumaran and Perkinson, 2000; Vestgarden, 2001) across climatic gradients and differing soil N status under natural conditions (Michel and Matzner, 2003). However, most of these studies were carried out on nutrient limited temperate forests of North Europe rather than tropical forests, while little is known on microbial activity within the C-cycle in forest soils under Mediterranean climate. Recently, the interest has

moved particularly towards litter decomposition processes in Mediterranean forests (Fioretto et al., 2005; Alarcón-Gutiérrez et al., 2008) but less knowledge about the response of microorganisms to a change of inorganic N_{in} in these soils is available. Although Mediterranean ecosystems are usually characterized by a small soil organic C-pool compared to northern European forest, these areas are *per se* susceptible to changes due to external disturbance of climatic and anthropogenic nature; hence, it is important to understand if a possible alteration of the C cycle may occur due to increased N availability.

The objective of this work is to investigate i) the impact of a simulated slow increase of N_{in} on the microbial performances of a Mediterranean forest soil; ii) how the microbial response to N_{in} availability might consequentially affect soil C-turnover and iii) if and how this response depends on fertilizer amount and incubation time, by medium of a 4 month incubation experiment.

We hypothesized that our approach might offer a new perspective in the study of microbial responses to N availability in soil. Laboratory conditions allow a major control on C and N dynamics by way of: investigation of microbial performances using independent measures of C-mineralization and C-substrate utilization *via* the analyses of community level physiological profile (CLPP) and enzymes, which related the microbial activity with its functional diversity. Moreover the laboratory approach excluded most limiting factors such as moisture and temperature conditions, and other nutrients availability, thus allowing the discrimination of the pure N_{in} effect, only. Lastly, what is new, compared to other similar incubation experiments, is the supply of N_{in} in small aliquots throughout the experiment, which allowed us to mimic a gradual increase of soil N status. In most studies, in fact, N was supplied in a single dose at the beginning of incubation (Södeström et al., 1983; Thirukkumaran and Parkinson, 2000; Michel and Matzner, 2003; Alarcón-Gutiérrez et al., 2008) or added with time but avoiding N_{in} accumulation (Magill and Aber, 2000; Vestgarden, 2001).

2.2. Materials and methods

2.2.1 Site characteristics and sampling

Soil samples were collected at the Roccarespampani forest site (Viterbo, Central Italy, 42.40 Lat, 11.93 Long). The forest, a coppice of *Quercus cerris* L. 19 years old, is characterized by a Mediterranean climate with an average annual temperature of 14 °C and an average annual rainfall of 755 mm. The forest is subject to coppice with a rotation cycle of 20 years and the soil, classified as Chromic Luvisol, shows an organic layer of 4 cm, on average. More detailed information on the coppice characteristics are reported in Tedeschi et al. (2006).

The composite samples were collected in three different points, corresponding to the three profiles opened in the site, covering a total area of 150 m^2. To investigate the effect of increasing N_{in}

availability in the late stage of organic matter decomposition, the soil samples were collected in the organic layer, including soil intimately mixed with decomposed plant residues (Tedeschi et al., 2006). Plant material still recognizable was removed by hands picking and consequently the soil was sieved at 2 mm and stored at -4 °C until the experimental set-up was terminated. Subsamples were taken to determine the soil properties reported in Table1.

Fig.1A Organic layer at the sampling site

Fig.1B Forest floor after organic layer removed

TOC	mg C g^{-1}	15 (0.11)
TN	mg N g^{-1}	1.15 (0.053)
C/N		13 (0.51)
pH		6.7 (0.05)
biomass-C	µg BC g^{-1}	1818.8 (198.5)
N-NH4	µg N-NH4 g^{-1}	20.15 (4.7)
N-NO3	µg N-NO3 g^{-1} l	14.21 (1.17)
mineral P	µg P g^{-1}	4.80 (0.48)

Table 1 Initial soil features of the investigated soil samples (fresh soil). In brackets the standard deviation (n=3) is reported.

2.2.2 Experimental design

Soil samples were pooled together with the aim to minimize any interference due to the intrinsic heterogeneity of the site. Samples were then put in plastic beakers and preconditioned at 28 °C and 60% of the water holding capacity (WHC) 24 hours before starting the incubation. Different amounts of nutrient solution were added dissolving in deionized water inorganic N in the form of ammonium-nitrate salt (NH_4NO_3). The samples were treated with the equivalent of 0, 10, 25, 50 and 75 kg N ha^{-1} (hereafter named N0 the control, N1, N2, N3 and N4 group) corresponding to a final dose of 0, 0.3, 0.7, 1.3 and 2 mg N g^{-1}, values comparable with the amount of fertilizer added in previous experiments in literature. Control soil samples were treated with an equal amount of deionized water. In the present experiment we simulated a slow and a more realistic increase of soil N status supplying the total dose of NH_4NO_3-N in small constant aliquots during the incubation

period. With this purpose we tried to limit an initial osmotic shock for microorganisms and allowed microbial community to deal with a slow change in nutrient and chemical status of soil. Each single aliquot was manually added once a week; the amount of nutrient solution added to soil samples was chosen depending on the average water loss of the sample in one week at 28 °C.

Since the aim of the experiment was to investigate the effect of N_{in} only, the NH_4NO_3 solutions were prepared adding KH_2PO_4 in ratio N:P = 5:1 in order to prevent an a priori nutrient limitation effect satisfying the stoichiometrical microbial need (Allison and Vitousek, 2005). N-solutions were corrected to soil pH with NaOH 0.1 M to avoid any substrates-induced pH effect on microbial activity. All samples, prepared in three replicates, were stored for 4 months in a thermostatic chamber at 28 °C and at 60% WHC allowing soil microbes to work at optimal conditions. The weight of each sample was recorded at the beginning of the treatment and soil moisture was recovered twice a week with deionized water in order to maintain soil moisture between 55% and 60%WHC.

2.2.3 C and N variables

Total organic C, total N and the microbial biomass C and N were measured in three replicates for the different N_{in} treatments at the beginning and at the end of incubation (t0 and t_{end} groups, respectively). Total C and N were determined by dry combustion (Thermo Soil NC - Flash EA1112), while the C and N in microbial biomass were determined using the fumigation-extraction method (Vance et al., 1987; Brookes et al., 1985) with 0.5 M K_2SO_4 as extracting solution and a conversion coefficient k_{ec}=0.64 for computing biomass-C. Microbial biomass-N was analyzed in the K_2SO_4 extracts following Joergensen and Brookes (1990) measuring ninhydrin-reactive nitrogen in fumigated and not-fumigated samples. The not-fumigated K_2SO_4-extractable carbon values were used as a proxy of the extractable dissolved organic C (DOC) (Allison et al., 2008). Mineral N was determined in triplicates at the beginning of the incubation, prior to each addition, at day 6, 13, 26, 55, 83 and at the end of the experiment, extracting 10 g of soil with 1 M KCl for ammonium (NH_4-N) and 0.5 M K_2SO_4 for nitrates (NO_3-N) and then analyzed colorimetrically according to Anderson and Ingram (1993) and Cataldo et al. (1975). C-mineralization was measured at day 1,3,7 and then weekly in three replicates for each experiment. 10g of soil aliquot were incubated in sealed jars using an alkali trap for CO_2 (Horwath and Paul, 1994). The excess of NaOH was titrated with 0.1 N HCl. Cumulative mineralization was computed summing the amount of C respired during the week. The metabolic quotient (qCO_2) was calculated as the amount of C-CO_2 produced per unit of microbial biomass C while the microbial quotient ($q$mic) was computed as the ratio between microbial biomass-C and the total organic C measured at the end of the experiment. The metabolic quotient is an index of the physiological ststus of microbial biomass, i.e.

a higher metabolic quotient means microorganisms have to use a higher aliquot of their C stock as energy in order to maintain a suitable physiological level under stress condition (Anderson and Domsch 1990). The pH of the samples was determined in three replicates in water with a ratio soil solution of 1:2.5:, while the moisture content was determined on oven dried (105 °C) samples .

2.2.4 Enzymatic activities

Microbial enzymes are responsable for soil organic matter degradation and contribute to the cycling of different nutrients within the ecosystem. Soil microbial enzymatic process and its understanding within context of external disturbance is hence of paramount importance and was indicated as a useful tool to asses soil quality, functioning and capability of the natural system to recovery an external perturbation. Moreover the enzymatic approach, although the limitations exposed in the introduction, is a suitable method to upscale the ecological functionality expression within the microbial community that is constituted by several species and families.

Eight hydrolytic enzymes and one oxidative enzyme were determined at the beginning of the incubation, at day 6, 13, 26, 55, 83 and at the end of incubation as a proxy of microbial performance in terms of organic matter decomposition and substrates utilization. The activity of the following hydrolytic enzymes was tested: β-glucosidase (bG), α-cellobiohydrolase (CEL), β-xylosidase (XYL) and α-glucosidase (aG) are key C-enzymes involved in cellulose, hemicellulose and starch degradation thereinafter considered as labile-C; N-acetyl-β-glucosaminidase (NAG) and leucine-aminopeptidase (LEU) are involved in N cycling through the release of amino sugars from chitin (Miller et al., 1998) and amino acids from polypeptides respectively; acid phosphatase (AP) is a crucial enzyme involved in organic P transformation; butyric esterase (BUT) is an endocellular enzyme indicated as an indirect parameter for the presence of active biomass (Wittman et al., 2004). The hydrolytic enzymes activities were measured *via* a fluorimetric approach (Marx et al., 2001) using 96 wells microplates and model substrates that return as products 4-methylumbelliferone (MUF) or 7-amino-4-methylcoumarin (AMC). The activities were expressed as unit of nmol of product g^{-1} dry soil h^{-1}. For each sample 1 g of fresh soil was weighed and homogenized with an Ultra-Turrax (IKA) in 50 ml acetate buffer (pH 6.1) for 3 min at 9500 rev min^{-1}. An aliquot of 100 μl 1 mM of each substrate was added to 50 μl of the homogenized suspension and 50 μl of acetate buffer. Reference standard wells received 50 μl of soil suspension to give a final amounts of 0, 50, 100, 200, 400, 600 and 800 pmol MUF or AMC $well^{-1}$.The plates were incubated for three hours at 28 °C and the fluorescence was read at fixed intervals (excitation 360 nm; emission 450 nm) with an automated fluorimetric plate-reader (Fluoroskan Ascent).

Due to the difficulties to discriminate a specific oxidative activity, we measured the polyphenol oxidases following the procedure of Perucci (2000). The enzymatic assay evaluates the o-diphenol

activity (OXI) that includes both tyrosinases and laccases (Burke and Cairney, 2002). These enzymatic families are involved in depolymerization and polymerization of complex substances as lignin and lignin-like compounds. The analytical method is a whole-soil method that follows the oxidation of catechol to quinone and the reaction with proline that gives a red compound. The activity is expressed as μmol cathecol oxidated 10 min^{-1} g $soil^{-1}$. Soil samples of 1 g were incubated for 10 minutes at 30 °C with a mixture of 1.5 ml of 0.2M catechol as substrate and 1.5 ml 0.2M of proline and 2 ml of 0.1 M phosphate buffer (pH 6.5). The reaction was stopped in an ice bath after addition of 5 ml of ethanol. The samples were then centrifuged at 4 °C at 8000 rpm. The absorbance of the new product in the supernatant was measured at 525 nm and the concentration computed using an extinction coefficient equal to 5.0 x 10^3. The control is represented by the oven-dried samples (105 °C) to measure the abiotic chemical oxidation and, hence, evaluate for difference the biotic component of the oxidative activity.

All assays were executed in three replicates for each N_{in} treatment and three analytical replicates. Data analysis was performed on instantaneous enzymatic rates (EA) and on cumulative activity (E) in order not to lose information throughout the experiment. The enzymatic investment E was computed by integrating enzymatic rates over time (i.e. the total incubation period); Ec is the total enzymatic investment for the decomposition of polysaccharides including bG, aG, CEL and XYL; analogously En, and Eox refer respectively to decomposition of organic N, including both NAG and LEU, and recalcitrant C. The ratio between the different terms is computed and normalized to control soil, in order to detect the percentage change as N_{in} dose increases. Thus the ratio between Ec and Eox is a proxy of the total investment of microbial community towards the acquisition of labile C against investment towards less decomposable C-compounds (Sinsabaugh et al., 2002). Shannon´s diversity index ($H'=-\sum p_i \log 2 p_i$) was computed on enzymatic rates as a measure of functional diversity (Bending et al., 2004) where p_i is the ratio of the activity of a particular enzyme to the sum of activities of all enzymes. Shannon diversity index was computed on standardized values to data of the first day of incubation.

2.2.5 Community level physiological profile (CLPP)

The catabolic response profile (CRP) of microbial community initially proposed by Degens and Harris (1997) is an approach that assesses the catabolic diversity involved in the process of substrate use adding different carbon source. This type of investigation is usefull since include indirectly several informations such as microbial status (basal respiration), catabolic activity, potential for nutrient cycling etc. CLPPs studies include thus C utilization pattern of soil microbial community testing the capability to metabolize different organic C-substrates (Degens and Harris, 1997). The fingerprint of microbial response to different soil N_{in} status was determined using the

MicroResp method (Campbell et al., 2003) where simple organic compounds are added directly to soil and short respiration responses are determined after six hours of incubation at 28 °C. The method has been used in several field experiments, in organic and mineral forest soil (Campbell et al.2003, Lalor et al.2007, Chapman et al. 2007) and in peatland soils (Artz et al.2006) showing the capability to discriminate soils or treatments using few C-substrates compared with other methos such as the CRP in Degens and Harris (1997) or the Biolog method (Campbell et al.1997, Chapman et al.2007). The advantage of MicroResp method compared to BIOLOG is the inclusion of the relatively slower growing organisms because it is a whole soil method and because the lower substrate concentrations do not support the growth of r-strategists.

MicroResp analysis was performed at the beginning and at the end of the incubation. C-substrates for the analysis of CLPP were selected depending on their ecological relevance and the objective of the experiment in order to improve the discrimination power.

The guild-sources included five carbohydrates (α-D-glucose, N-acetyl-Glucosamine, D-Galactose, D-fructose, L-arabionose), five amino acids (L-leucine, L-arginine, Glycine, L-aspartic acid and γ-amino-butyric acid), three carboxylic acids (citric acid, oxalic acid and L-ascorbic acid) and two phenolic acids (vanillic and syringic acid). The latter two substrates are commonly found in decaying plant residues, soils and root (Whitehead et al.1983), and have been chosen to be representative of the decomposition of the more recalcitrant organic matter. Each C-substrate was dissolved in deionized water to deliver from 30 to 0.75 mg C g soil water^{-1} (Table 2).

SUBSTRATES	**LABEL**	concentration mg C g^{-1} soil w
D-glucose	G	30
N-acetyl-D-gluc.	NAG	7.5
D-galactose	GA	30
D-fructose	F	30
L-arabinose	ARA	30
L-leucine	LEU	3
Glycine	GLY	7.5
L-arginine	ARG	7.5
γ-amino-butyric acid	BUT	30
L-aspartic acid	ASP	0.75
L-ascorbic acid	ASC	30
Citric acid	CIT	30
Oxalic acid	OX	7.5
vanillic acid	VAN	0.3
syringic acid	SYR	0.3

Table 2 Substrates selected for CLPP analysis with MicroResp method. Final concentration of substances expressed as mg carbon per g of soil water are reported.

Substrate concentrations were chosen from values previously utilized in other CLPP studies with MicroResp method (Campbell et al., 2003, Lalor et al., 2007). For some substances the molarity of the solution was adjusted depending on their solubility and concentration into the soil, so to avoid toxicity for microbial cells (Fulthorpe and Allen 1994).

A subexperiment was carried on at t0 on fresh soil treated without N addition (control) and adding total N4 dose in one single addition with and without pH correction. The samples were incubated 24 hours at 28°C.

MicroResp method worked as a multi-SIR; a detection plate positioned over the deep-well plate with soil, was filled with a gel that changes colour due to the acidification process once the CO_2 produced by microorganisms reacted with the sodium carbonate in the gel mixture (Fig.2). Colour development of the indicator was measured as absorbance (ABS) at 590 nm using a Biolog MicroStation (Microlog^{TM} Release 4.20.04, Biolog, Inc., Hayward, CA). Measured values were then converted to % CO_2 using a least square fitting curve on experimental data that relates absorbance measured to % CO_2 of equilibrium with gel after 6 hours of incubation.

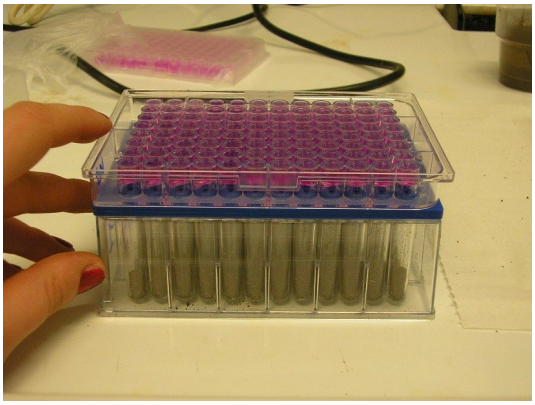

Fig. 2 MicroResp method: detection plate and deepwell plate filled with soil and C-substrates.

The calibration curve was determined on the experimental data reported in Campbell (2003), since we performed the CLPP in the same experimental conditions and with the same experimental setup (i.e. same deepwell and detection plates with the same volume of reference). Moreover we considered that several type of soils were considered for the purpose and that were characterized by a large spectrum of respiration rates, making more reasonable to use the same set of data (Fig. 3). Data were fitted following the rectangular hyperbole: % CO_2 =-0.3409-1.4604/(1-7.882*ABS). Finally the data were converted to a flux of μg C- CO_2 g^{-1} soil h^{-1}.

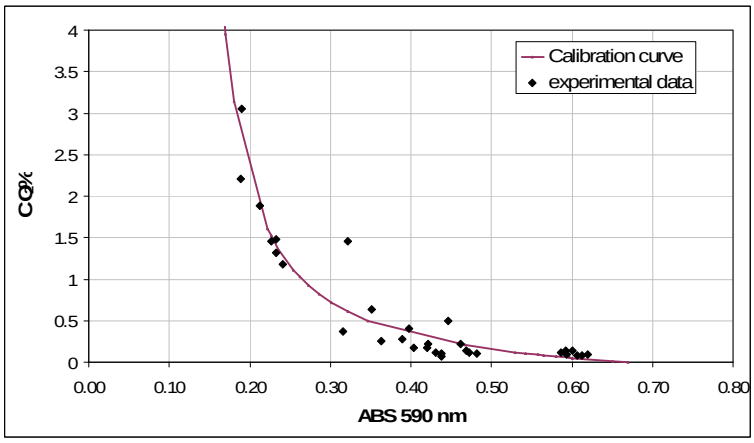

Fig. 3 Calibration curve for determining the concentration of CO_2 as % as function of absorbance measured at 590nm.

Multivariate analysis was performed on data after subtracting the control value assessed with the addition of deionized water and dividing by the mean respiration of samples of the substrates (Campbell et al., 2003).

2.2.6 Functional diversity

Ecosystem functioning is largely controlled by soil microbial dynamics that drive the carbon and nutrient cycling within the ecosystem (Nannipieri et al.2003, Caldwell 2005). Anthropogenic activity can alter both directly and indirectly the functioning and diversity of a natural system via land use changes or direct pollution because of N fertilization and N depositions and hence the maintenance of these functions is of paramount importance in order to conserve the soil health.

In the present work we tried to study the response to external disturbance ad N addition at the process level investigating the effect of the key drivers of some important processes such as the enzymatic-mediated processes without taking account for the dynamics within the community structure (i.e. the taxonomic characterization of the microorganisms). In fact the study of functional diversity is a way to get through the poor knowledge in terms of link between genetical, taxonomical diversity within the system under study. Functional diversity constitutes hence an approach to study the microbial diversity with a more ecological relevance (Zack et al.1994) until more knowledge about microbial diversity and the single biochemical process is gained. Functional diversity include the contribution of both reachness of enzymes produced rather than substrates used and the intensity of the microbial performance considering all the enzymatic rates and respiration of the different C compounds.

Shannon´s diversity index ($H'=-\sum p_i \log 2 p_i$) was computed on enzymatic rates as a measure of functional diversity (Bending et al., 2004) where p_i is the ratio of the activity of a particular enzyme to the sum of activities of all enzymes. Shannon diversity index was computed on standardized values to data of the first day of incubation. Shannon´s diversity index was computed analogously

as for enzymatic activities substituting the term p_i with the respiration rate of each single C-substrate as SIR (Degens et al., 2000). The index was computed on standardized values to data of the first day of incubation.

2.3 Statistical analyses

The main effect of N_{in} on biochemical variables was examined by analysis of variance (Repeated ANOVA) for time per N effect and at different times of incubation (one-way ANOVA) followed by the Fisher LSD test (Post hoc test). Correlations between the different variables are computed and reported using the Pearson's correlation statistic. CLPP and enzymatic discrimination capability among treatments was computed with the discriminant function analysis (DFA). DFA is a multivariate statistics that uses an a priori classification to maximize the power of separation of those variables that generate the greatest difference between the experimental groups. DFA was followed by determination of Mahalanobis distances for computing the effective separation of groups. All the statistics was elaborated with STATISTICA version 7.0 (data analysis software, Stat.Soft. INC.2004). For CLPP analysis with DFA we used only the respiration rates of the substrates that were consistently greater than the basal respiration response when only deionized water was added to the soil. For instance glycine and L-aspartic acid SIR were removed from the analysis.

2.4 Results

The preliminary chemical and biochemical analyses are reported in Table 1. Concerning the four months experiment, we split the correlations evaluation with regards to only the significant incubation time t26 and t_{end} , since all the variables measured during the incubation period showed a significant time*N effect (repeated ANOVA; $P<0.05$).

2.4.1 C – N pools and fluxes

Cumulative C-mineralization (R_{cum}) increased with N_{in} availability up to N3 treatment corresponding to 1.3 mg N g^{-1} added (Table 3). At the maximum dose of 2 mg N g^{-1}, N4 experiment showed a final value similar to control N0 and a lower and statistically different value from N3. At t26, cumulative respiration was not affected by N_{in} treatment (Table 4A) while at the end R_{cum} showed a positive correlation with N_{in} (Table 4B). C-mineralization rate expressed as hourly respiration (Fig. 4) exhibited comparable values between control soil and N1, N2 and N3 treatments in the first month of incubation; while after t26 the N1, N2 and N3 groups started to respire over control. From t80 to the end, the N3 group showed a slow increases of respiration rate differing from the other treatments and control soils. N4 group revealed a specific pattern: in the first month

of incubation microbial respiration responded negatively to fertilization showing a C-mineralization rate lower than control group ($P<0.01$). After t55 a sharp recovery occurred until t100 where a final depression of activity was measured.

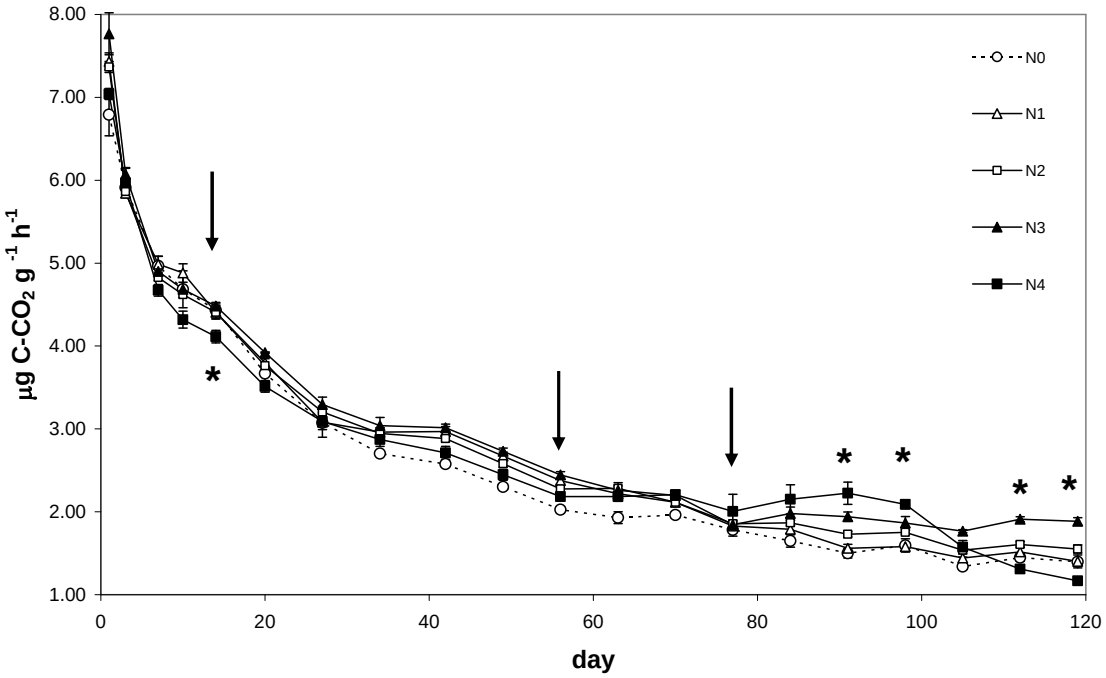

Figure 4 Hourly respiration for each treatment during incubation time. Asterisk denotes where N4 treatment is different from the other groups N0, N1, N2 and N3 ($P <0.05$; LSD test). Time*N effect is significant at $P<0.05$. The first two arrows refer to suppression and promotion of respiration in N4 group while the third arrow refers to the promotion of respiration in N3 group (see text for more detail).

The trend of N_{in} in soil solution in the form of ammonium and nitrate increased constantly along time (Figs. 5A, 5B). The mineral NH_4-N pool, after a small decrease in the first week, increased rapidly during incubation time while nitrates increased linearly. In control soils and N1 treatment NH_4-N increased slightly maintaining values below 20 µg NH_4-N while the highest N_{in} dose reached a final value of 250 µg NH_4-N. A significant nitrification process occurred since data referring to NO_3-N showed values an order of magnitude higher compared to ammonium. N4 treatment reached a final value of 1400 µg NO_3-N .

In correspondence of a value of 100 µg NH_4-N and 600 µg NO_3-N after t26, the N4 group started to respire over control soils although the differences were not significant. At t55 were a concentration of 200 µg NH_4-N and 800 µg NO_3-N was measured in N4 group, respiration rate increased over N1, N2 and N3 groups and then drastically decreased after t100. When N3 treatment had an N_{in} pool comparable to N4 at t55, it started to respire over groups N1 and N2 (Fig. 4).

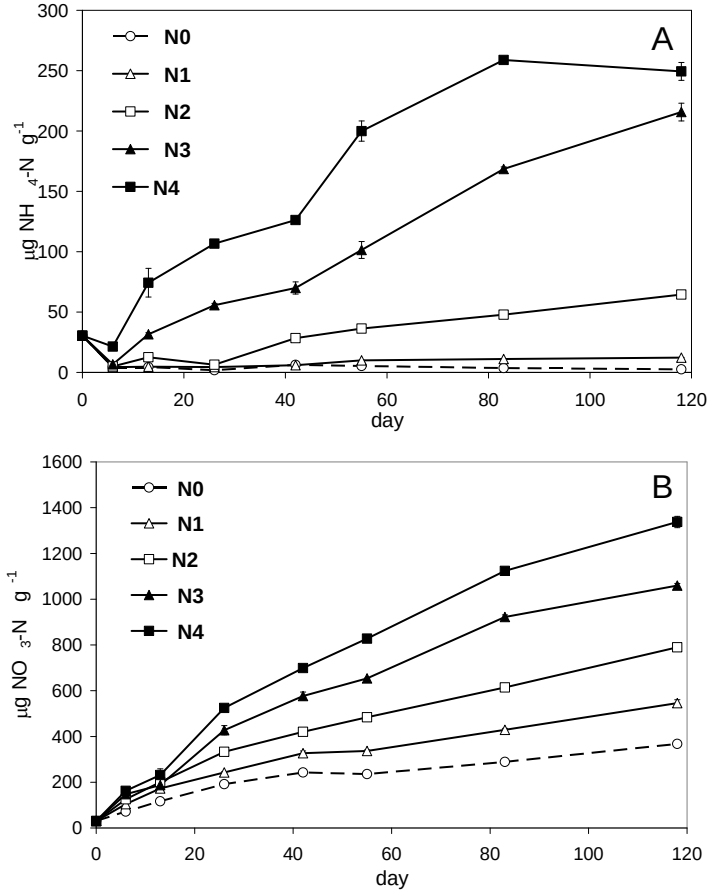

Figure 5: A) Extractable NH_4-N for each N treatment during the incubation period. B) Extractable NO_3-N for each N treatment during the incubation period.

N_{in} fertilization significantly affected the biochemical variables measured at the end of the incubation (Table 3). Soil microbial biomass decreased from N0 to N4 treatment ($P<0.01$) while extractable DOC showed a positive trend with fertilization ($P<0.01$) and the differences were highly significant (Table 3). Similarly to microbial biomass, the pH values decreased from 6.4 of control soil to 5.4 of N4 group (Table 3; $P<0.01$); analogously microbial biomass C to N ratio ranged from 10.9 in the control group to 4.7 in the N4 group (Table 3; $P<0.01$). Microbial indexes qCO_2 and $q$mic, respectively, increased and decreased significantly with N_{in} dose (Table 2; $P<0.01$).

Microbial biomass was negatively correlated to the final content of N_{in} (Table 4B; $P<0.01$) and positively to pH value at the end of incubation (Table 4B; $P<0.01$). DOC variables were positively related to N_{in} (Table 4B; $P<0.01$) while C to N ratio of biomass and soil pH values were negatively influenced by fertilization (Table 4B; $P<0.01$). TOC at t_{end} was negatively correlated with R_{cum} but did not exhibit any correlation with C-enzymes (Table 4B).

	TOC			TN			C-min cum			DOC			biomass-C			C/N biomass			qCO_2			q_{mic}			pH		
	%			%			$\mu g\ C\text{-}CO_2\ g_s^{-1}$			$\mu g\ C\ g_s^{-1}$			$\mu g\ C\ g_s^{-1}$						$g\ C\text{-}CO_2\ h^{-1} mg\ C_{mic}^{-1}$			%					
t0	15.3	(0.12)	a	1.09	(0.008)	a							1818.9	(199)	a	5.7	(0.5)	a				1.5	(0.0001)	a	6.7	(0.03)	a
N0	14.5	(0.07)	b	1.1	(0.003)	a	6797.6	(119)	a	265.8	(6)	a	2151.3	(17)	b	10.9	(0.3)	b	0.65	(0.01)	a	1.5	(0.0001)	a	6.4	(0.06)	b
N1	14.5	(0.18)	b	1.16	(0.014)	b	7217.3	(157)	b	314.6	(28)	a	1434.9	(73)	c	10.6	(0.4)	b	0.98	(0.04)	ab	1.0	(0.0006)	b	5.8	(0.03)	c
N2	14.2	(0.11)	bc	1.2	(0.014)	b	7290.4	(117)	cb	404	(11)	b	1252.4	(30)	c	6.0	(0.8)	a	1.24	(0.07)	bc	0.9	(0.0002)	bc	5.7	(0.03)	d
N3	13.8	(0.12)	c	1.25	(0.022)	c	7685.2	(107)	dc	394.4	(17)	b	1174.3	(44)	c	6.2	(0.9)	a	1.61	(0.05)	cd	0.9	(0.0003)	c	5.5	(0.01)	e
N4	14.6	(0.18)	b	1.41	(0.016)	d	7192.2	(135)	ab	557.1	(32)	c	635.3	(84)	d	4.7	(0.5)	a	1.93	(0.35)	d	0.4	(0.0006)	d	5.4	(0.02)	e

Table 3 Values at the beginning and at the end of incubation of TOC, TN, cumulative mineralization, DOC, biomass-C, C/N ratio of microbial biomass, metabolic quotient, microbial quotient and pH for different N treatments. Mean values and standard deviation within brackets are reported. Different letters denote significant ($P < 0.05$; LSD test) differences among treatments, n=3.

	NH_4-N	NO_3-N	R_{cum}
R_{cum}	ns	ns	
CELL	0.76	0.62	-0.56
bG	0.92	0.86	-0.55
aG	0.92	0.92	ns
XYL	0.90	0.79	ns
NAG	0.84	0.77	-0.55
AP	0.89	0.80	ns
BUT	0.63	0.54	-0.65
LEU	0.85	0.75	-0.66
OXI	-0.59	-0.54	0.55

Table 4A Pearson correlation coefficient r computed for the biochemical variables at t26 of incubation. Correlations are significant at $P< 0.05$.

	NH_4-N	NO_3-N	R_{cum}	pH	TOC	C-biomass	Biomass C/N	DOC
NH_4-N					ns	-0.81	-0.81	0.82
NO_3-N	0.96				ns	-0.92	-0.87	0.91
R_{cum}	0.51	ns			-0.61	ns	ns	ns
pH	-0.78	-0.87	-0.65		ns	0.93	0.79	-0.78
CELL	0.83	0.86	ns	-0.66	ns	-0.78	-0.61	-0.90
bG	0.73	0.68	ns	-0.52	ns	-0.62	ns	-0.74
aG	0.58	0.5	ns	ns	ns	ns	ns	ns
XYL	0.75	0.76	ns	-0.54	ns	-0.71	-0.63	-0.82
NAG	0.93	0.93	ns	-0.70	ns	-0.79	-0.70	-0.98
AP	0.78	0.84	ns	-0.62	ns	-0.79	-0.66	-0.89
BUT	ns	ns	-0.55	0.61	0.67	0.59	ns	ns
LEU	ns	ns	ns	ns	0.53	ns	ns	ns
OXI	ns	ns	ns	ns	0.5	ns	ns	ns

Table 4B Pearson correlation coefficient r computed for the biochemical variables at the end of incubation. Correlations are significant at $P< 0.05$.

2.4.2 Enzymes

Enzyme activities related to C-cycling exhibited different patterns among N_{in} doses and along incubation time. The C-acquiring enzymes have been split in hydrolytic enzymes involved in degradation of more labile substances (EAc) and the oxidative enzyme related to more recalcitrant compounds (EAox). EAc included thus the contribution of bG, aG, CEL and XYL (Fig. 3A). The different activities have been summed because they showed the same general pattern in response to time and N_{in} addition. On average, activities decline in the first 40 days, followed by a weak

increase and a reduction in the last month (Fig. 3A). However, a significant interaction between time and N treatment was observed ($p<0.01$). Indeed, N4 exhibited a separate pattern ($p<0.01$), with an initial 45% increase, a strong reduction at day t55, and a positive trend at the end of incubation (Fig. 3A). Unlike hydrolytic enzymes, oxidative activity showed a decline among time for all treatments (time x N $p<0.01$; Fig. 3B), although N4 treatment presented a weak diminution in oxidation rates at the beginning, followed by a slow increase after t83. Despite the mineral nutrients (N and P) supplied in the treatment solution, NAG and AP activities exhibited a significant positive trend throughout the experiment and with N_{in} particularly for N2, N3 and N4 groups ($p< 0.01$; Fig. 3C-E) while N0 and N1 did not manifest an important pattern. LEU showed a response similar to EAc although less markedly (time x N $p<0.01$; Fig. 3D) with a diminution of rates in all the groups. BUT activity revealed a similar behaviour to that of the hydrolytic C-enzymes; in particular the enzymatic rate for the highest N_{in} dose increased by 25% after the first week and a decline followed at t55, while control soil and the other treatments dropped smoothly (time x N $p<0.01$; Fig. 3F).

After one month bG, aG, CEL, NAG, BUT and LEU were negatively correlated to cumulative respiration (Table 4A), while at the end of incubation only BUT maintained this negative correlation to R_{cum} (Table 4B). Most of the hydrolytic enzymes showed at t26 and at t_{end} a positive relation to N_{in} (Table 4A-B; $P< 0.05$) in particular with NH_4-N; BUT and LEU did not exhibit any correlation to N_{in} at t_{end} (tab.4B). With the exception of BUT, the hydrolytic enzyme activities were negatively correlated to pH, to biomass C and DOC (Table 4B). Markedly, BUT was positively correlated to microbial biomass-C (Table 4B). OXI activity showed a negative correlation to N_{in} at t26 (Table 4A; $P< 0.05$) losing any correlation to N_{in} towards the end. Throughout the incubation period the overall oxidative activity decreased significantly being the enzyme mostly associated to hourly respiration ($r=0.81$, $P<0.01$; data not shown).

The ratio between Ec and Eox decreased slightly up to N2 with a decrement equal to 7%, and then increased significantly in N4 group by 30% when compared to control (Table 5; $P<0.01$). Considering the instantaneous values, the pattern of the ratio EAc to EAox followed the same similar behaviour along incubation time (data not shown). The ratio Ec to En was not affected by N_{in} treatment although a slow decrease can be observed between experimental groups (Table 5). The parameter R_{cum}/Ec increased with N_{in} dose until N3 and then decreased for the highest dose to a value lower than control (Table 5; $P<0.01$).

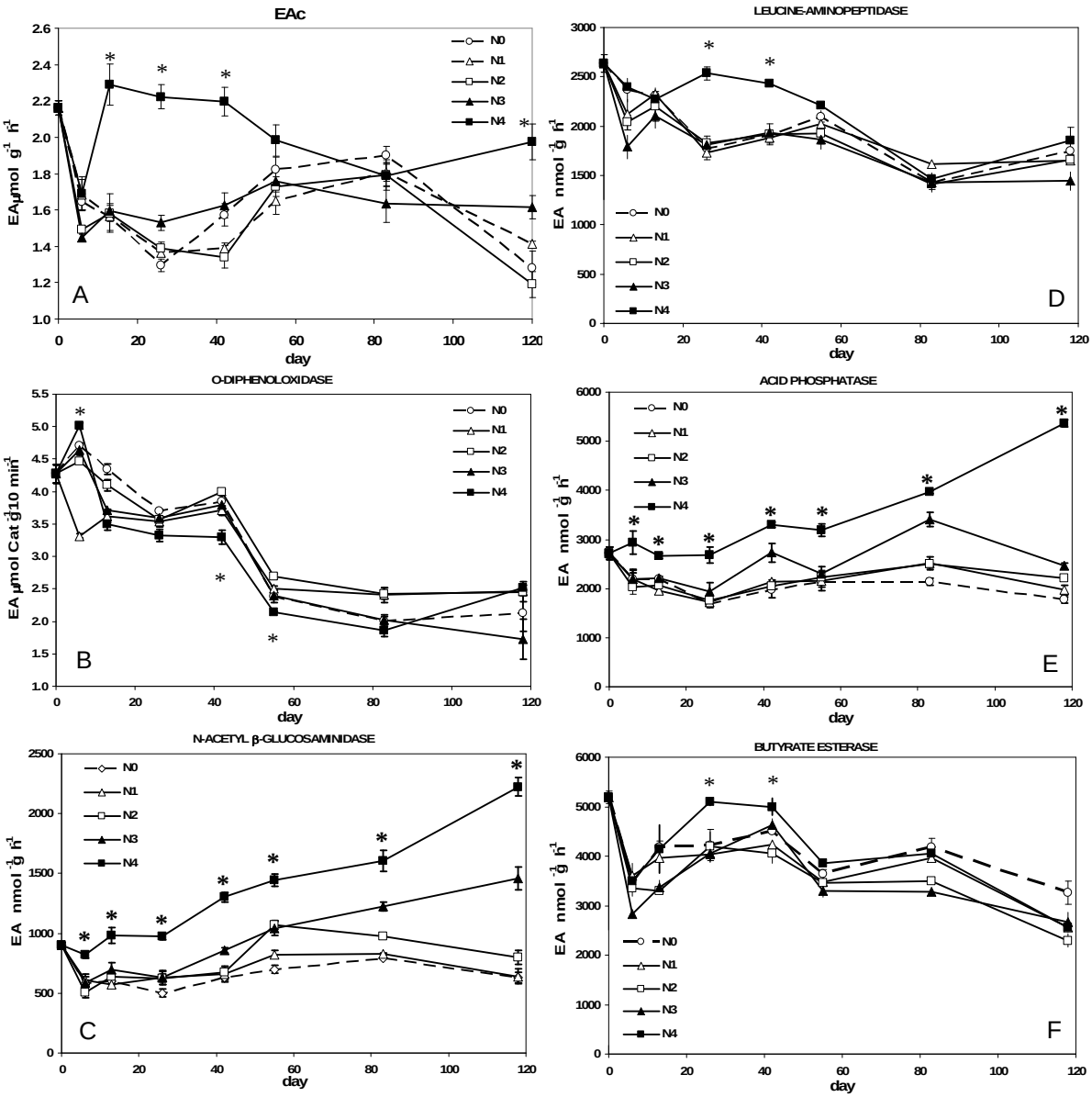

Figure 6: Pattern of the enzymatic activity of A) hydrolytic C-acquiring enzymes (including β -glucosidase, α-glucosidase, cellulase and β-xylosidase); B) o-diphenoloxidase; C) N-acetyl-β-glucosaminidase; D) leucine-aminopeptidase ; E) acid-phosphatase; F) butyrate-esterase. Asterisks denote where N4 treatment is different from the other groups N0, N1, N2 and N3 ($P < 0.05$; LSD test). Time*N effect is significant at $P<0.01$ for all the enzymes.

When computing Ec against En for each time of measurement a linear pattern can be detected concerning the enzymatic investment that is conservative among treatment and incubation time (Fig. 7).

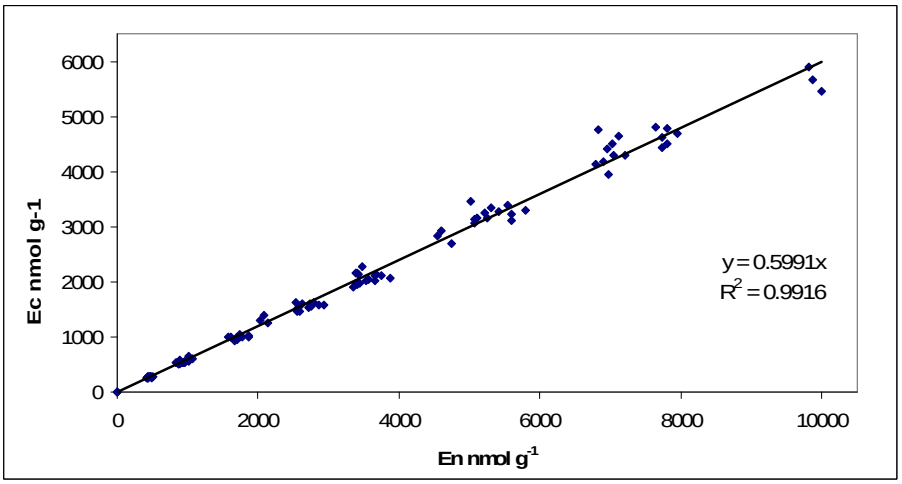

Figure 7: Cumulative enzymes Ec and En computed at the different time of incubation.

	R_{cum}/E_C	E_C/E_N	E_C/E_{OX}
N0	1.00 a	1.00 a	1.00 ab
N1	1.09 ab	0.95 a	1.04 ab
N2	1.13 b	0.91 a	0.93 b
N3	1.13 b	0.91 a	1.04 ab
N4	0.87 c	0.88 a	1.30 c

Table 5 R_{cum} refers to cumulative respiration at the end of incubation, enzymatic activities refer to enzymatic rates integrated over time. Ec is the total enzymatic investment for the decomposition of polysaccharides including bG, aG, CEL and XYL; analogously En and Eox refer respectively to the decomposition of organic N, including both NAG and LEU, and recalcitrant-C. Ratios are normalized with respect to control group. Different letters denote significant ($P < 0.05$; LSD test) differences among treatments, n=3.

At the end of incubation, the microbial functional diversity index was negatively affected by addition of N_{in} ($P<0.01$) decreasing from 3.14 for N0 to 2.93 for N4 (Fig. 7); including time effect, t0, N0 and N1 had a similar Shannon index. The treatment influenced H' index throughout incubation (N*Time $P<0.001$): the N_{in} fertilization increased slightly but significantly at $P<0.05$ the diversity index of N4 group in the first month of incubation and then the values of N2, N3 and N4 treatments decreased drastically close to the end (Fig. 6).

The DFA was performed on enzymatic activities including the data at t0. The multivariate analysis detected two significant roots explaining a total variance between groups of 90%; the analysis returned a clear separation particularly between groups belonging to sub experiments N3, N4 and t0 group as reported in fig. 4A. N3 and N4 groups were discriminated from N0, N1 and N2 (Mahalanobis distance: $P<0.05$) while there were no differences within the treatments N0, N1 and

N2. CEL, bG, NAG and AP were the enzymes mostly contributing to discrimination (Table 6) with a negative correlation to the root 1 (which explained 62% of variance). The other enzymes were correlated to the second root (which explained 28% of variance); LEU, BUT and OXI were the enzymes showing the highest correlation coefficients (Table 6). Considering all treatments it seemed that N_{in} fertilization negatively shifted the groups towards root 1, hence to a higher CEL, NAG and P- acquisition activity. Moving from N0 to N3 a negative dislocation occurred along root 2 increasing correlation of groups with this root, hence in particular towards a lower activity of BUT and OXI.

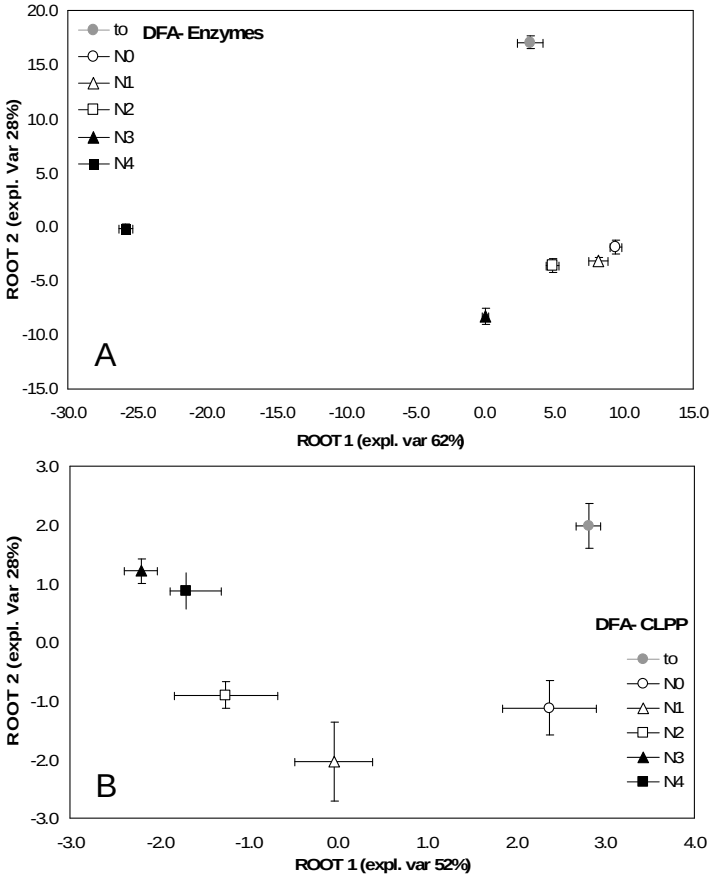

Figure 8: DFA analysis of A) enzymatic rates and B) SIR data from MicroResp method at the beginning of incubation t0 and at the end of the incubation for N0, N1, N2, N3 and N4 group. Roots are significant at $P<0.05$.

	ROOT1 (62%)	ROOT2 (28%)
CEL	**-0.77**	**0.53**
bG	**-0.48**	**0.63**
aG	-0.18	**0.80**
XYL	-0.41	**0.82**
NAG	**-0.95**	-0.11
AP	**-0.98**	0.13
BUT	0.19	**0.90**
LEU	-0.03	**0.95**
OXI	0.02	**0.93**

Table 6 Correlations of variables (enzymatic rates) to the two roots of the DFA analysis applied to enzymatic data (marked correlations are significant at P <0.05).

2.4.3 Community level physiological profile

The catabolic activity of microorganisms at the beginning and at the end of the experiment were clustered for the different classes of substrates (Fig. 9). The mean respiration rate for each C-source group was statistically different for N3 and N4 treatment compared to the other groups which showed a comparable substrates use. In particular SIR data revealed a lower utilization of carbohydrates, amino acids and carboxylic acids ($P<0.05$) with increasing N_{in} availability, while no differences in aromatic substances utilization were detected. For N1 and N2 treatments a higher respiration of carboxylic acid was observed. The CLPP performed on soil samples treated with the total N4 dose with and without pH correction, showed a comparable suppression respect to the control soil, neverless the corrected N4 solution exhibited a lower inhibition (Fig. 10).

DFA was computed for CLPP data at the beginning and at the end of incubation using all the standardized respiration rates of the single C-compounds divided by the mean respiration in order to consider the differences between microbial community of each group (Campbell et al., 2003). The first root explained the 52% of variance between groups, while the second root explained the 28% of variance (Fig. 8B). Compared to enzymatic data, the substrates utilization rates revealed a weaker discrimination power between treatments; a differentiation exist between t0 and fertilized soils ($P<0.01$), but an overlapping of groups was showed within samples analyzed at the end of incubation. Most of carbohydrates, γ-amino butyric acid and L-ascorbic acid were correlated with root1 while the other substrates were correlated with the second root in expecially glucose and syringic acid (Table 7). Despite the low capability of discrimination a trend can be observed from N0 to N4 group with N_{in} addition, moving negatively along root 1, hence, towards a general lower utilization of carbohydrates and amino-acids.

Functional diversity computed as Shannon index decreased weakly with N_{in} fertilization from 3.51 in N0 group to 3.41 in N4 group (Fig. 12; $P<0.05$) although indexes of N2, N3 and N4 were statistically comparable.

	ROOT1 (52%)	ROOT2 (28%)
D-glucose	**0.41**	**-0.75**
N-acetyl-D-gluc.	**0.40**	-0.27
D-galactose	0.28	-0.30
D-fructose	**0.50**	-0.28
L-arabinose	**0.55**	-0.31
L-leucine	**0.49**	0.05
L-arginine	-0.17	**-0.52**
γ-amino-butyric acid	**0.42**	**-0.36**
L-ascorbic acid	**0.36**	-0.06
Citric acid	0.05	**-0.46**
Oxalic acid	-0.01	**-0.39**
vanillic acid	0.04	-0.21
syringic acid	0.19	**-0.66**

Table 7 Correlations of variables (substrates) to the two roots of the DFA analysis applied to SIR data (marked correlations are significant at $P<0.05$).

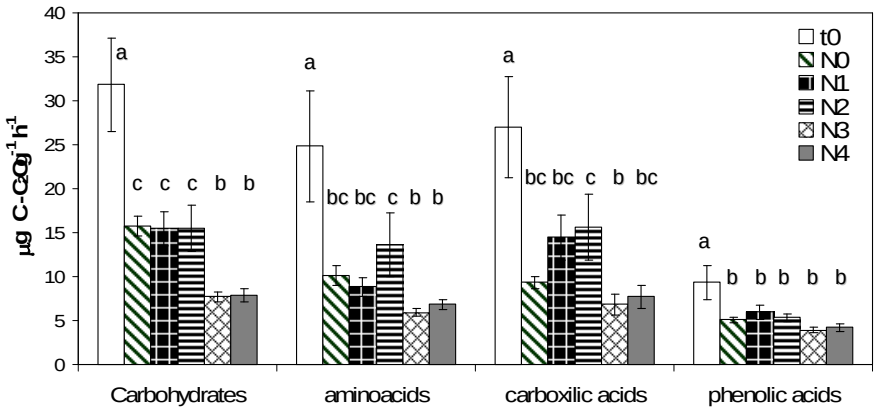

Figure 9 CLPP for classes of C-substrates among N treatments expressed as µg C respired as CO_2, different letters denote significant ($P<0.05$; LSD test) differences among soil groups; n=3.

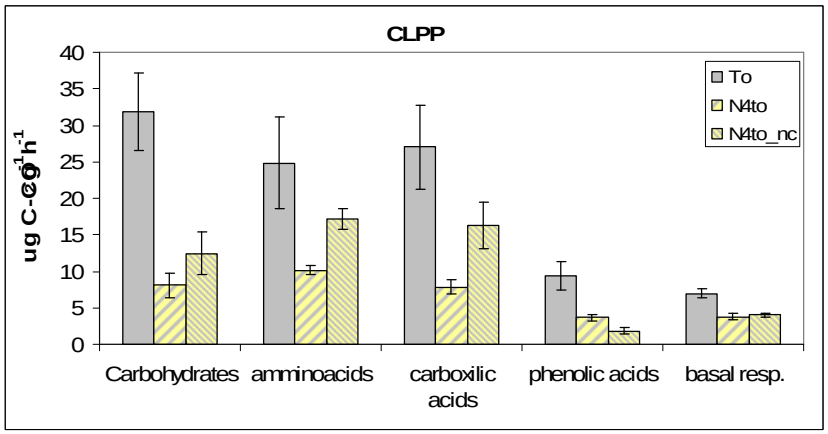

Figure 10 CLPP performed on fresh soil at t0 after 24 hours of incubation without N addition (To), and with addition of total N4 dose with (N4to) and without pH correction (N4to nc). Data are expressed as µg C respired as CO_2, different letters denote significant (P <0.05; LSD test) differences among soil groups; n=3.

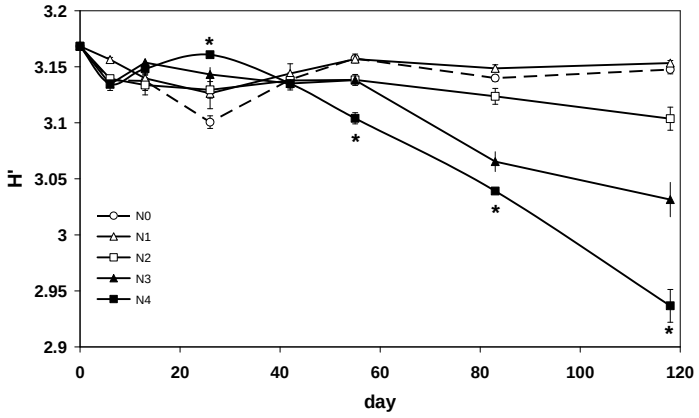

Figure 11 Pattern of Shannon index H' for enzymatic activity throughout the incubation time. Time*N effect significant at P<0.001. Asterisks denote where N4 treatment is different from the other groups N0, N1, N2 and N3 (P <0.05; LSD test).

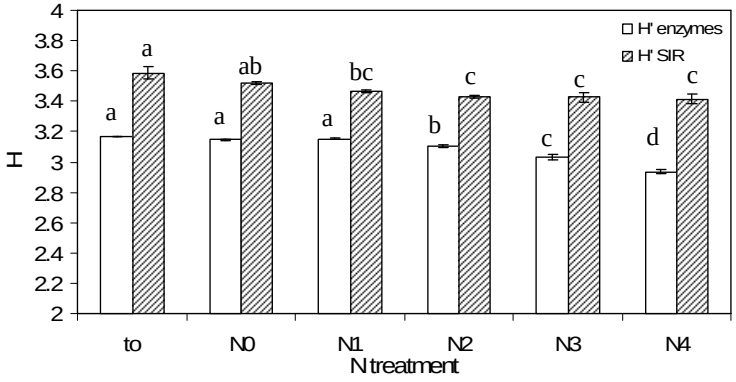

Figure 12 Shannon index H' for enzymatic data and MicroResp data at the beginning and at the end of incubation, different letters denote significant (P <0.05; LSD test) differences among soil groups; n=3.

2.5 Discussion

The soil preliminary analysis showed a total N content of 1.15 % with a C/N ratio of 13, an almost low value if compared to the fertilization studies conducted in other experiments, where C/N ratio ranged between 18 and 40 in the organic layer (Michel and Matzner, 2003; Waldrop et al., 2004; Pregitzer et al., 2008).

Concerning the N_{in} pools, NH_4-N showed a small diminution after the first week and a slow increase during incubation, while N-NO_3 increased linearly reaching a much higher final value, suggesting the prevalence of a nitrification process. The initial decrease of ammonium might suggest a preferential immobilization of this form by microorganisms limited to the first week (Lagomarsino et al., 2008). Almost 65% of the total amount of added N was recovered in the soil solution - the missing fraction may be attributable to several mechanisms including volatilization and transformation in organic form. However in this work we focused on the effect of the mineral N effectively present in the soil on the microbial activity. In particular the aim was to highlight the microbial functions in order to emphasize the ecological role of soil microbes.

The slow increase of N_{in} availability in the four months incubation of the Mediterranean forest soil, led to a microbial response which exhibited a specific behaviour that depended on the incubation time and fertilizer amount, particularly behaviour pertaining to C degradation and nutrient acquisition mechanisms. A non linear response of soil microbial activity to N_{in} addition has been reported in other studies (Berg and Matzner, 1997; Michael and Maztner, 2003; Allison et al., 2009) but in the present experiment, the responses changed with the incubation time, depending on the soil N_{in} concentration and on the incubation time at which that concentration has been reached. In particular the N4 group revealed an independent behaviour with regards to both C-mineralization and enzymes compared to the other fertilization treatment. To denote the temporal differences of microbial responses to N_{in} addition, we split the results in two phases: the first month of incubation and the following months of the experiment.

2.5.1 First month of incubation

In the first month of incubation N_{in} did not influence treatments N1, N2 and N3, while for N4 soil the amount of 300-500 $\mu g\ g^{-1}$ N_{in}, reached within the 3rd week, had a suppressive effect on C-mineralization, thus reducing C loss. Concurrently the hydrolytic enzyme activities involved in C-cycling were strongly promoted in N4 samples while no significant effects occurred in the other groups. The significant increase of butyrate esterase (an indicator of active biomass, Withmann et al., 2004), and C degrading enzymes (EAc), coupled to the suppression of respiration in the N4 group, suggested, for the first month, a temporary redirection of C-pathways from energy-spilling to

investment in biomass or enzymes. These findings would support the mechanism proposed by Schimel and Weintraub (2003) of a redirection of C-resources by microbial community depending on specific needs and energetic issues. In addition the N4 group exhibited a small but significant increase of the Shannon index (Fig. 6; $p<0.05$) approaching t_{26}, The higher functional diversity of N4, concurrent with the observed pattern of C-enzymatic rates and the increased butyrate-esterase activity, supported the hypothesis that N4 dose had a positive effect on microbial communities at the beginning of the incubation.

In constrast, during the first two months oxidative activity was suppressed, suggesting a lower utilization of the more recalcitrant C. This shift away from the recalcitrant substances under N_{in} addition has been indicated as a negative priming by Blagodastakaya and Kuzyakov (2008) and may be the mechanism justifying the lower oxidative activity detected in the N4 group. Although the oxidative activity has been indicated to be more favoured in alkaline soils (Sinsabaugh et el., 2008), a biological mechanism may be more reasonable compared to a chemical effect linked to an acidification process. In fact the solution added was corrected to soil pH and the amount of N_{in} added during the first month was low, thus excluding a direct effect of soil pH. The promotion of the C-enzymatic activity, while suppressing the oxidative activity in N4, occurred along all the incubation time. Moreover while for N0, N1, N2 and N3 samples differences in Ec/Eox ratio were less evident, the N4 group exhibited the highest Ec/Eox ratio. This behaviour follows the trend of other experiments performed *in-situ* under oak forest litter and mineral soil (Carreiro et al., 2000; Sinsabaugh et al., 2002, 2005). Thus, although the experiment was carried out in a laboratory under optimal conditions and without plants, we were able to observe an enzymatic response similar to studies performed in fertilized oak forests under different climatic conditions. Our findings would thus be in accordance with the suggestion that, in the late stage of decomposition, recalcitrance seems to modulate the response to N addition more than climatic factors (Coûteaux et al., 1995; Johansson et al.2005).

During decomposition processes an initial preference for the breakdown of compounds with a higher return (in terms of energy and low-molecular weight polymers) may be reasonable (Chiellini et al., 2007). Hence, we may hypothesize that the occurrence of a labile C pool at the beginning of experiment probably made the microbial community more sensitive to N_{in} addition, but only in the N4 group. Consequently, it is possible that, although the soil under investigation had a significant background level of N, this was not all in an easily available form for the microbial community, thus affecting the microbial utilization of soil C (Hagedorn et al., 2003; Craine et al., 2007; Lagomarsino et al., 2007; Blagodastakaya and Kuzyakov, 2008). Moreover nutrient availability can not only regulate the activity but may act as a regulator for species selection (Fontaine et al., 2003;

Sinsabaugh et al., 2008), making the interpretation of microbial response under N fertilization more difficult.

2.5.2 Second, third and fourth month of incubation

Once N_{in} availability rose after the first month, a general stress condition occurred for all the treatments compared to the control soil with a promotion of respiration for groups N1, N2 and N3, whereas no significant C-enzymatic stimulation took place. In contrast to what was observed for the N4 group, C-mineralization was not suppressed in the N3 and N2 groups once they had a N_{in} content of 500 μg N-NH_4NO_3, highlighting how the rate of N_{in} addition affected the response of microbial community.

The same amount of N_{in} added in a longer period was not likely to promote a negative priming effect or the growth of a guild feeding on more labile substances in N2 and N3 groups. Finally, when the N4 and N3 groups had an inorganic N pool close to 1000 μg NH_4NO_3-N, at t55 and t80 respectively, they started to increase respiration, suggesting that this amount of N_{in} is likely to be a critical load for microbial activity. For N4 treatment close to a content of 1000 μg NH_4NO_3-N the enhanced C-mineralization was concomitant with a strong decline in C-enzymatic activity, probably indicating a selective pressure due to N_{in}.

In fact, in time, the slow increase of N_{in} availability altered negatively the physiological status and hence the functionality of microorganism groups in all the treated soils as underlined by the Shannon index. The results obtained from both microbial pools and enzymatic pattern, suggested, hence, a negative pressure mechanism rather than a shift in microbial species composition with N_{in} addition that was most pronounced for N4 group and toward the end of incubation. The occurrence of a general stress condition at t_{end} was further supported by either the increase of qCO_2 or the decrease of *q*mic. Besides that, the DFA analyses showed a lower utilization of carbohydrates with N_{in} and a trend toward higher NAG and AP activity.

In fact NAG activity increased almost monotonically with time and with N_{in} treatment. In other studies, NAG activity has been reported either to decrease (Waldrop et al., 2004; Waldrop and Zack, 2006) or increase (Sinsabaugh et al., 2002; Saiya-Cork et al., 2002; Michel and Matzner, 2003) under N_{in} fertilization in field and laboratory incubation of forests. Hence the response of this enzyme has not been universal. This positive trend could be justified by the release of chitin due to fungal cell lysis. Chitin supplies both labile N and C: hence, this could be an advantage for microbes under stress, supporting a preference for this substrates; in fact LEU did not follow the same pattern of NAG although releasing labile organic N. Concurrently, since βG is mainly produced by fungi (Miller et al., 1998) its pattern could indicate a strong decline in fungal community due to the occurred stressed state, occurrence, a mechanism that was relevant to all the

treatments approaching the end of experiment. To some extent the almost constant Ec/En ratio throughout time and between groups would suggest that N_{in} addition may have partially satisfied microbial N demand while microorganisms continued to assimilate N in organic form, in particular from chitin (Cohen–Kupiet et al., 1998). The pattern of cumulative Ec against En computed at the different day of measurement supports our statement as well.

2.5.3 End of incubation

The pattern of microbial performances in the last period of incubation time affected the final values of C pools. N_{in} fertilization negatively influences the microbial biomass C pool at t_{end}, according to other studies performed in laboratory (Södeström et al., 1983; Tirukkumaran and Perkison, 2000) or field conditions (DeForest et al., 2004; Compton et al., 2004; Demoling et al., 2008), although in some cases no changes in total microbial community have been detected (Michael and Matzner, 2003). Anderson et al. (2004) found that in European forests, along a climatic gradient from Sweden to Italy, biomass-C decreased with N depositions in the humus layer. Besides the direct effect of increasing N_{in} availability, in this study the decrease of microbial biomass C was strongly correlated to soil pH. The enhancement of biological nitrification processes with N_{in} addition contributed to the pH decrease. Nevertheless, at N4 dose, the pH was similar to N3, whereas microbial biomass C strongly decreased, suggesting a biological effect of N_{in} availability on microbial community, larger than chemical inhibition of pH (Södestrom et al., 1983; Tirukkumaran and Parkinson, 2000). Microbial biomass C to N ratio also showed a linear decrease with increasing N_{in} doses, reflecting increased N_{in} availability to microbes. Moreover, compared to the C/N ratio at t0, samples treated with low N_{in} doses showed a lower N accumulation per unit of assimilated C, while for experiments with doses higher than for N1, a shift occurred towards a microbial community requiring more N (Hodge et al., 2000).

The decrease of microbial biomass is not supported by a corresponding decrease of DOC, which was positively affected by increasing N doses. Extractable-DOC concentration increased with N_{in} availability in agreement with other studies (Sinsabaugh et al., 2004; Waldrop and Zack, 2006), although DOC was found to be suppressed in other experiments (Sjöberg et al., 2003; Demoling et al., 2008) or unaffected in some litter incubation studies (Vestgarden, 2001; Magill and Aber, 2000).We can hypothesize that: i) dead microbes contributed to DOC enrichment; ii) DOC was partly constituted by more recalcitrant compounds of phenolic origin (Waldrop and Zack, 2006). This second hypothesis is supported by the oxidative activity trend. In fact o-diphenolase decreased significantly during incubation for all soils and treatments as indicated by DFA on enzymatic rates, suggesting that labile C was not high enough to support the degradation of recalcitrant C (Allison and Vitouseck, 2005). In addition, as showed by CLPP analysis, the weakening capability of

microbial biomass to use carbohydrates and amines with increasing N_{in} doses, coupled with a similar use of phenolic compounds among samples, seems to indicate that microorganisms are mostly feeding on recalcitrant compounds.

The decline of respired CO_2 observed with CLPP with N doses higher than N2 at the end of incubation is in agreement with the observed stress condition and with the strong reduction of functional diversity (of both enzyme and CLPP), as well as with the increase of metabolic quotient. The decreased functional diversity with N_{in}, computed using enzymatic activity, corresponded with the trend toward a reduced utilization of all the substrates as detected with CLPP analysis, thus altering the efficiency of the system.

However, understanding of the coupling of changes in enzymatic activity and organic matter degradation processes is still far from complete due to the broad range of enzymes within the single microbial specie and the potential high redundancy of function within the total community (Degens et al., 2000; Nannipieri et al., 2003; Caldwell, 2005). On the other hand, loss of functional diversity may result in a potential increase of soil vulnerability with respect to external disturbance that is crucial in ecosystems under Mediterranean climate.

The point of most remarkable interest is that the final value of measured TOC does not follow the trend of mineral N, despite that the other final C-pools and biochemical variables showed a clear correlation with final N_{in} concentration. In fact, the N4 group exhibited a higher C content compared to N3, which revealed the highest C loss. The amount of TOC at the end of incubation reflected the pattern of microbial respiration, as shown by the negative correlation between the two. In fact, the cumulative respiration of the N4 group is comparable to the value for the control soil and was not strongly stimulated by N_{in} addition, whereas N1, N2 and N3 showed increased C loss with N_{in}. This pattern found its correspondence in the computed cumulative enzymatic effort (Ec) and cumulative respiration ratio (R_{cum}). Actually, Ec is the effort needed to decompose the more labile C that can quickly provide energy and C for synthesis of new biomass or enzymes. Hence R_{cum} /Ec ratio can be interpreted as a measure of the investment in enzymatic substrate degradation of labile compounds against C-loss as respiration-- namely an index of how the system is able to reinvest C for its own needs. The N1, N2 and N3 groups showed increased R_{cum}/Ec ratio, whereas it was lower in the N4 treatment, thus suggesting that soil nutrient *status* had apparently favoured, on average over the entire period, the capacity of the microbial community to increase the predominance of anabolic over catabolic functions (Thiet et al., 2006). Markedly, with respect to other studies, these findings highlight how the initial biological effect of increasing mineral N had an important fingerprint on the highest N treated samples, despite the relative low amount of N_{in} accumulated in the first month.

2.6 Conclusion

In the present experiment, the gradual slow N_{in} increase clearly influenced microbial response in Mediterranean forest soil, consequently affecting soil C dynamics. Expanding upon other studies in N-limited forests, the soil incubation experiment for its particular setting highlighted how soil response may be highly dependent on the dynamics of microbial community and its adaptation capability: soil microbes changed their functions in relation to the temporal scale of the processes resulting in a non- linear response to N_{in} addition through time and for varying N_{in} doses. Of note, the increase of microbial C use efficiency under low increases of N availability induced a potential accumulation of C in the system in the short-term. Over a longer period, the promotion of C loss from the system occurred at higher doses, due to stress conditions and a decrease of functional diversity. A comparison of the short-term results with field experiments performed under the same vegetation but under other climatic conditions (Carreiro et al., 2000; Sinsabaugh et al., 2002; Sinsabaug et al., 2005), adds confidence to the existence of an ecosystem-dependent framework, such that the biological response to increasing N availability on C cycling in soil follows a similar behaviour for the same type of ecosystem, thus overshadowing direct climate control.

However, further investigation is necessary to robustly assess the control of inorganic N on soil organic matter decomposition in forest soils (including interaction with plants and in particular with labile-C due to fresh litter), with the ultimate aim of effectively quantifying the potential loss or gain of C.

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References

Alarcón-Gutiérrez, E., Couchaud, B., Augur, C., Calvert, V., Criquet, S., 2008. Effects of nitrogen availability on microbial activities, densities and functional diversities involved in the degradation of a Mediterranean evergreen oak litter (*Quercus ilex* L.). Soil Biology & Biochemistry 40, 1654–1661.

Allison, S.D., Vitousek, P.M., 2005. Responses of extracellular enzymes to simple and complex nutrient inputs. Soil Biology & Biochemistry 37, 937–944.

Allison, S.D., Czimczik, C.I., Treseder, K.K., 2008. Microbial activity and soil respiration under nitrogen addition in Alaskan boreal forest. Global Change Biology 14, 1156–1168.

Allison, S.D., LeBauer, D.S., Ofrecio, M.R., Reyes, R., Ta, A., Tran, T., 2009. Low levels of nitrogen addition stimulate decomposition by boreal forest fungi. Soil Biology & Biochemistry 41, 293–302.

Anderson, T.H., Domsch, K.H., 1990. Application of eco-physiological quotients (qCO_2 and qD) on microbial biomasses from soils of different cropping histories. Soil biology & biochemistry 22, 251-255.

Anderson, J.M., Ingram, J.S.I., 1993. Colorimetric determination of ammonium. In: Anderson, J.M., Ingram, J.S.I. (Eds.), Tropical Soil Biology and Fertility, A Handbook of Methods. second ed. Anderson, CAB International, Wallingford, U.K, pp. 73–74.

Andersson, M., Kjøller, A., Struwe, S., 2004. Microbial enzyme activities in leaf litter, humus and mineral soil layers of European forests. Soil Biology & Biochemistry 36, 1527–1537.

Arzt, R.R.E., Chapman, S.J., Campbell, C.D., 2006. Substrate utilisation profiles of microbial communities in peat are depth dependent and correlate with whole soil FTIR profiles. Soil Biology & Biochemistry 38, 2958–2962.

Bending, G.D., Turner, M.K., Rayns, F., Marx, M-C., Wood, M., 2004. Microbial and biochemical soil quality indicators and their potential for differentiating areas under contrasting agricultural management regimes. Soil Biology & Biochemistry 36, 1785-1792.

Berg, B., Matzner, E., 1997. Effect of N deposition on decomposition of plant litter and soil organic matter in forest ecosystems. Environ. Rev. 5, 1-25.

Blagodatskaya, E., Kuzyakov, Y., 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. Biology and fertility of soil 45, 115-131.

Burke, R.M., Cairney, J.W.G., 2002. Laccases and other polyphenol oxidases in ecto- and ericoid mycorrhizal fungi. Mycorrhiza 12, 105–116.

Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biology & Biochemistry 17, 837-842.

Caldwell, B.A., 2005. Enzyme activities as a component of soil biodiversity: A review. Pedobiologia 49, 637-644.

Campbell, C.D., Chapman, S.J., Cameron, C.M., Davidson M.S., Potts, J.M., 2003. A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of microbial communities by using whole soil. Applied and Environmental Microbiology 69, 3593-3599.

Campbell, C.D., Grayston, S.J., Hirst, D.J., 1997. Use of rizosphere carbon sources in sole carbon source test to discriminate soil microbial communities. Soil Biology & Biochemistry 30, 33-41.

Carreiro, M.M., Sinsabaugh, R.L., Repert, D.A., Parkhurst, D.F., 2000. Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. Ecology 81, 2359–2365.

Cataldo, D.A., Haroon, M., Schrader, L.E., Young, V., 1975. Rapid colorimetric determination of nitrate in plant tissue by nitration of salycilic acid. Commun. Soil Sci. Plant Anal. 6, 71–80.

Chapman, S.J., Campbell, C.D:, Artz, R.R.E., 2007. Assessing CLPPs using MicroResp. A comparison with Biolog and multi-Sir. J. Soil Sediments 7,406-410.

Chiellini, E., Corti, A., D'Antone, S., Billingham, N.C., 2007. Microbial biomass yield and turnover in soil biodegradation tests: carbon substrate effects. Journal of Polymers and the Environment 15, 169-178.

Cohen-Kupiec, R., Chet, I., 1998. The molecular biology of chitin digestion. Current Opinion in Biotechnology 9, 270-277.

Compton, J.E., Watrud, L.S., Porteous, L.A., DeGrood, S., 2004. Response of soil microbial biomass and community composition to chronic nitrogen addition at Harvard forest. Forest Ecology and Management 196, 143-158.

Coûteaux, M., Bottner, P., Berg, B., 1995. Litter decomposition, climate and litter quality. Tree 10, 63-66.

Craine, J.M., Morrow, C., Fierer, N., 2007. Microbial nitrogen limitation increases decomposition. Ecology 88, 2105-2113.

DeForest, J.L., Zak, D.R., Pregitzer, K.S., Burton, A.J., 2004. Atmospheric Nitrate Deposition, Microbial Community Composition, and Enzyme Activity in Northern Hardwood Forests. Soil Sci. Soc. Am. J. 68, 132–138.

Degens, B.P., Harris, J.A., 1997. Development of a physiological approach to measuring the catabolic diversity of soil microbial communities. Soil Biology & Biochemistry 29, 1309-1320.

Degens, B.P., Schippera, L.A., Sparlinga, G.P., Vojvodic-Vukovi, M., 2000. Decreases in organic C reserves in soils can reduce the catabolic diversity of soil microbial communities. Soil Biology & Biochemistry 32, 189-196.

Demoling, F., Nilsson, L.O., Bååth, E., 2008. Bacterial and fungal response to nitrogen fertilization in three coniferous forest soils. Soil Biology & Biochemistry 40, 370–379.

Fioretto, A., Di Nardo, C., Papa, S., Fuggi, A., 2005. Lignin and cellulose degradation and nitrogen dynamics during decomposition of three leaf litter species in a Mediterranean ecosystem. Soil Biology & Biochemistry 37. 1083–1091.

Flaig, W., 1971. Organic compounds in soil. Soil Science 111, 19-33.

Fontaine, S., Mariotti, A.,, Abbadie, L., 2003. The priming effect of organic matter: a question of microbial competition? Soil Biology & Biochemistry 35 (2003) 837–843.

Fulthorpe, R.R:, Allen, D.G., 1994. Evaluation of Biolog MT plates for aromatic and chloroaromatic substrate utilization tests. Canadian journal of microbiology 40, 1067-1071.

Galloway, J.N., Aber, J.D., Erisman, J. W., Seitzinger, S. P., Howarth, R. H., Cowling, E. B., Cosby, B.J., 2003. The nitrogen cascade. Bioscience 53, 341-356.

Gundersen, P., Emmett, B.A., Kjønaas, O.J., Koopmans, C.J., Tietema, A., 1998. Impact of nitrogen deposition on nitrogen cycling in forests: a synthesis of NITREX data. Forest Ecology and Management 101, 37-55.

Hagedorn, F., Spinnler, D., Siegwolf, R., 2003. Increased N deposition retards mineralization of old soil organic matter. Soil Biology & Biochemistry 35, 1683–1692.

Hodge, A., Robinson, D., Fitter, A., 2000. Are microorganisms more effective than plants at competing for nitrogen? Trends in plant science 5, 304-308.

Horwath, W.R., Paul, E.A., 1994. Microbial biomass, in: Weaver, R.W., Angle, S., Bottomely, P., Bezdicek, D., Smith, S., Tabatabi, A., Wollum, A. (Eds.), Methods of Soil Analysis, Part II. Microbiological and Biochemical Properties. Soil Science Society of America,Madison, Wisconsin, pp. 753–773.

Hungate, B.A., Dukes, J.S., Shaw, M.R., Luo, Y., Field, C.B., 2003. Nitrogen and Climate Change 302, 1512.

Knorr, M., Frey, S.D., Curtis, P.S., 2005. Nitrogen additions and litter decomposition: a meta-analysis. Ecology 86, 3252-3257.

Joergensen, R.G., Brookes, P.C., 1990. Ninhydrin-reactive nitrogen measurements of microbial biomass in 0.5 M K_2SO_4 soil extracts. Soil Biology & Biochemistry 22, 1023–1027.

Johansson, M.B., Berg, B., Meentemeyer, V., 1995. Litter mass loss rates in late stage of decomposition in a climatic transect of pine forests. Long-term decomposition in a Scots pine forest. IX. Can. J. Bot. 73, 1509-1521.

Lagomarsino, A., Knapp, B.A., Moscatelli, M.C.,, De Angelis, P., Grego, S., Insam, H., 2007. Structural and Functional Diversity of Soil Microbes is Affected by Elevated [CO2] and N Addition in a Poplar Plantation. J Soils Sediments 7, 399 –405.

Lalor, B.M., Cookson, W.R., Murphy, D.V., 2007. Comparison of two methods that assess soil community level physiological profiles in a forest eco system. Soil Biology & Biochemistry 39, 454–462.

Macdonald, J.A., Dise, N.B., Matzner, E., Armbruster, M., Gundersen, P., 2002. Nitrogen input together with ecosystem nitrogen enrichment predict nitrate leaching from European forests. Global Change Biology 8, 1028-1033.

Magill, A., Aber, J.D., 2000. Dissolved organic carbon and nitrogen relationships in forest litter as affected by nitrogen deposition. Soil Biology & Biochemistry 32, 603-613.

Magnani, F., Mencuccini, M., Borghetti, M., Berbigier, P., Berninger, F., Delzon, S., Grelle, A., Hari, P., Jarvis, P.G., Kolari, P., Kowalski, A.S., Lankreijer, H.L., Law, B.E., Lindroth, A., Loustau, D., Manca, G., John, B., Moncrieff, J.B., Rayment, M., Tedeschi, V., Valentini, R., Grace, J., 2007. The human footprint in the carbon cycle of temperate and boreal forests. Nature 447, 848-852.

Marx, M.C., Wood, M., Jarvis, S.C.,2001. A microplate fluorimetric assay for the study of enzyme diversity in soils. Soil Biology and Biochemistry 33, 1633-1640.

Melillo, J.M., Steudler, P.A., Aber, J.D., Newkirk, K., Lux, H., Bowles, F.P., Catricala, C., Magill, A., Ahrens, T., Morrisseau, S., 2002. Soil warming and carbon-cycle feedbacks to the climate systems. Science 298, 2173–2176.

Michel, K., Matzner, E., 2003. Response of enzyme activities to nitrogen addition in forest floors of different C-to-N ratios. Biol. Fertil. Soils 38, 102–109.

Miller, M., Palojärvi, A., Ranger, A., Reeslev, M., Kjøller, A., 1998. The Use of Fluorogenic Substrates To Measure Fungal Presence and Activity in Soil. Applied and environmental microbiology 64, 613–617.

Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G., Renella, G., 2003. Microbial diversity and soil functions. European Journal of Soil Science 54, 655–670.

Perucci, P., Casucci, C., Dumontet, S., 2000. An improved method to evaluate the *o*-diphenol oxidase activity of soil. Soil Biology & Biochemistry 32, 1927–1933.

Reay, D.S., Dentener, F., Smith, P., Grace, J., Feeley, R.A., 2008. Global nitrogen deposition and carbon sink. Nature Geoscience 1, 430-437.

Rustad, L.E., Campbell, J.L., Marion, G.M., Norby, R.J., Mitchell, M.J., Hartley, A.E., Cornelissen, J.H.C., Gurevitch, J., 2001. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental eco system warming. Oecologia 126, 543–562.

Saiya-Cork, K.R., Sinsabaugh, R.L., Zak, D.R., 2002. The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil ,2002. Soil Biology & Biochemistry 34, 1309–1315.

Schimel, J.P., Weintraub, M.N., 2003. The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biology & Biochemistry 35, 549–563.

Sinsabaugh, R.L., Carreiro, M.M., Repert, D.A., 2002. Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. Biogeochemistry 60, 1–24.

Sinsabaugh, R.L., Gallo, M.E., Lauber, C., Waldrop, M.P., Zak, D.R., 2005. Extracellular enzyme activities and soil organic matter dynamics for northern hardwood forests receiving simulated nitrogen deposition. Biogeochemistry, 75: 201–215.

Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosta, A.L., Cusack, D., Frey, S., Gallo, M.E., Gartner, T.B., Hobbie, S.E., Holland, K., Keeler, B.L., Powers, J.S., Stursova, M., Takacs-Vesbach, C., Waldrop, M.P., Wallenstein, M.D., Zak, D.R., Zeglin, L.H., 2008. Stoichiometry of soil enzyme activity at global scale. Ecology letters, 11: 1252–1264.

Sjöberg, G., Bergkvist, B., Berggren, D., Nilsson, S.I., 2003. Long-term N addition effects on the C mineralization and DOC production in mor humus under spruce. Soil Biology & Biochemistry 35, 1305-1315.

Södeström, B., Bååth, E., Lundgren, B., 1983. Decrease in soil microbial activity and biomasses owing to nitrogen amendments. Can. J. Microbiol. 29, 1500-1506.

Tedeschi, V., Rey, A., Manca, G., Valentini, R., Jarvis, P., Borghetti, P., 2006. Soil respiration in a Mediterranean oak forest at different developmental stages after coppicing. Global Change Biology 12, 110–121.

Tirukkumaran, C.M., Perkinson, D., 2000. Microbial respiration, biomass, metabolic quotient and litter

decomposition in a lodgepole pine forest floor amended with nitrogen and phosphorous fertilizers. Soil Biology & Biochemistry 32, 59-66.

Thiet, R.K., Frey, S., Six, J., 2006. Do growth yield efficiencies differ between soil microbial communities differing in fungal:bacterial ratios? Reality check and methodological issues. Soil Biology & Biochemistry 38, 837-844.

Van Bruggen, A.H.C., Semenov, A.M., 2000. In search of biological indicators of soil health and disease suppression. Applied Soil Ecology 15, 13-24.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil biology & biochemistry 19, 703–707.

Vestgarden, L.S., 2001. Carbon and nitrogen turnover in the early stage of Scots pine (*Pinus sylvestris L.*) needle litter decomposition: effects of internal and external nitrogen. Soil biology & biochemistry 33, 465-474.

Waldrop, M.P., Zak, D.R., Sinsabaugh, R.L., 2004. Microbial community response to nitrogen deposition in northern forest ecosystems. Soil Biology & Biochemistry 36, 1443–1451.

Waldrop, M.P., Zack, D.R., 2006. Response of Oxidative Enzyme Activities to Nitrogen Deposition Affects Soil Concentrations of Dissolved Organic Carbon. Ecosystems 9, 921–933.

Whitehead, D.C., Dibb, H., Hartley, R.D., 1983. Bound phenolic compounds in water extract of soils, plant roots and leaf litter. Soil Biology & Biochemistry 15, 133-136.

Wittmann, C., Kähkönen, M.A., Ilvesniemi, H., Kurola, J., Salkinoja-Salonen, M.S., 2004. Areal activities and stratification of hydrolytic enzymes involved in the biochemical cycles of carbon, nitrogen, sulphur and phosphorus in podsolized boreal forest soils. Soil Biology & Biochemistry 36, 425–433.

Zack, J.C., Willig, M.R., Moorhead, D.L., Wildman, H.G., 1994. Functional diversity of microbial communities: a quantitative approach. Soil Biology & Biochemistry 26, 1101-1108 .

3. LITTER AND SOIL ORGANIC MATTER DECOMPOSITION MODEL

Keywords: *modelling, soil N availability, Carbon-Nitrogen feedbacks, C stocks*

3.1 Introduction

The soil is a complex and open system where several physical, chemical and biological reactions take place (Brady and Weil 1999). This occurs both below the surface and at the interface with the atmosphere with very different temporal and spatial scales for each single process. Among the abiotic drivers of carbon (C) cycle such as temperature and moisture, soil nitrogen (N) status constitutes an important key to understand the biochemical processes of litter decomposition, soil organic matter (SOM) formation and thus C and N stabilization. The potential for soil C-sequestration due to increased N availability is a critical point in the contest of the role of forest ecosystems to mitigate global change effect (Nabuurs et al.2007; de Vries et al.2009), since on one hand soil, due to the low C/N, can immobilize less C compared to woody compartment with a high C/N. On the other hand, soil has the potential for immobilization of C and N in recalcitrant form for long time. However the direction and magnitude of the ecosystem response is still under investigation due to the high complexity governing soil and trees C sequestration (Janssen and Luyssaert 2009; Reay et al.2008; Sutton et al.2008).

The soil N availability, in particular in forest ecosystems, is modulated by two types of control. One is external as it is constituted by input as N deposition (Aber et al.2003; Galloway et al.2004) or N fertilization (i.e. used in the past to improve timber production in Northern Europe). The other contribution to increasing mineral N comes from the promoted SOM mineralization due to increasing soil temperature (Rustad et al.2001; Melillo et al.2002), releasing in turn more nutrients.

N deposition has greatly increased the N supply in several Central European and North American forests, ranging from 10 up to 75 Kg ha^{-1} y^{-1}(Gundersen et al., 1998; Macdonald et al., 2002) although this trend may be temporary (Shopps et al., 2003). The crucial issue concerns the fate of increasing mineral N input to forests and its pathways within the ecosystem once it has been buried . The soil, in particular microorganisms are the main responsible of N retention as organic matter (both as recalcitrant and proteinaceous form) rather than mineral (Nannipieri et al.2003; Schulten and Schnitzer 1998; Aber et al.1998) and of N turnover in the ecosystem, modulating its availability concurrently with plant competition for nutrient uptake, allowing the aliquot remaining to leach or being lost in gaseous form. In turn, any perturbation of these N-processes affects plants response in terms of growth and stock of C in the woody biomass since the balance between immobilization of N in the SOM and in plant tissues and the OM mineralization releasing again the nutrients is disturbed. The results is that plants exhibit a response to N deposition or increasing atmospheric CO_2 depending on the soil N status and the pathway of added N (Reich et al.2006; Nadelhoffer et al.1999; Hyvönen et al.2008).

The other control of internal nature is constituted by the concentration of N in the litter. Once deposed on the soil, its decomposition constitutes the first step toward soil C sequestration. Actually there is experimental evidence that increased input of N lead to elevated foliar N concentration (Magill et al.2004; Pregitzer et al.2008; Mallert et al.2004), and to increased litter fall and turnover (Reich et al.1999) regulating indirectly C cycling in the soil, affecting the microbial process of litter decomposition. All this mechanisms, among others such as the plant species composition, indirectly driven by climate change and N deposition, influences the structure, composition and spatial distribution of the microbial decomposer community (De Santo et al. 2002).

Research studies of litter and SOM decomposition under N fertilization has revealed a complex set of feedbacks between C and N dynamics. For instance the first step toward soil C sequestration and stabilization is litter decomposition; the effect of internal and external N depends on the stage of degradation and age of C compounds. Several studies in the literature have investigated the control of N on biological processes, and provided evidences that mineral N promotes litter decomposition on short temporal scale (fresh and N-poor litter) while inhibiting decomposition on late stage of decomposition, thus on old litter and humus (Fog 1988; Berg and Matzner, 1997) that accumulates in deeper soil layers. Although its chemical characterization is still far from being fully understood, humus can for instance be referred to as the final stage of litter decomposition before important physical and other chemical processes occurs at the interface with the mineral matrix. Moreover, according to various studies, recalcitrance seems to have an important role in mediating the soil response to N addition while climatic drivers have a minor role in controlling humus decomposition rate (Berg and Meentemayer 2002, Couteaux 2005, Knorr et al.2005). On the other side higher levels of internal N in litter may lead to an anticipated N mineralization (Manzoni et al.2008), since higher initial N leads to a more rapid initial decomposition followed by a more rapid decrease of litter quality affecting the accumulation of organic matter (Berg and Meentemayer 2002).

While respiration and microbial biomass are the variables monitored to investigate microbial activity in laboratory incubation experiments,, in the case of field fertilization experiments, the soil net C balance includes plant litter input and DOC leaching. Although the plant litter is promoted under N fertilization, it has been shown in some studies that the detected increased SOC was partially imputable to increased litterfall (Pretziger et al.2008, Franklyn et al 2003, Hyvönen et al.2008), but a significant contribution is justified by a reduction of microbial community activity, i.e. lower SOM decomposition (Neff et al.2002; Franklyn et al 2003; Hagedorn et al.2003). In fact different hypotheses have been reportedin the last 20 years following the pioneer work of Fog (1988) trying to explain the microbial response as a combination of biochemical effects that can involve ecological functions and community succession. Indeed, the mechanisms proposed in the litterature can be briefly summarized as an ecological selection toward a bacterial dominated system

(Sinsabaugh et al.2005; Frey et al.2004; Demoling et al.2008). This happens through the control of the quality of C compounds due to abiotic and biotic reactions of mineral N and amino compounds with recalcitrant C (Berg et al.1998: Magill and Aber 1998; Demoling et al.2008), the effect of inhibition on microbial performances, in terms of decomposition and anabolic C-pathway, and at the community level, structure and functions, expressing . Although N has the potential for soil acidification, and thus inhibition of microbial activity, it has been shown that the decrease of pH can not be regardedas the only factor affecting decomposition processes, since N has also a negative influence in originally acid soils (Magill et al. 1997, Tirukkumaran and Parkinson 2000) and continues after chemical effect has finished (Sodestrom et al.1983).

The response of soil microorganisms to variation of N availability is thus still under debate (and the mechanisms involved unknown) all the more so since it shows a non linear response both in lab incubation experiment and in field experiments. The reasons include several factors such as the diversity of ecosystems (Sinsabaugh et al.2002; Grandy et al.2008), the variable rates of fertilization (Knorr et al.2005) and the difficulties to perform long term N fertilized experiment and to detect significant changes in soil C stocks (Magill et al.2004, Pregitzer et al.2008). However, although a general scheme able to situate robustly the soil response to N addition does not exist yet, experimental evidences underline a trend toward a soil C-sink role (Reay et al.2008, chapt. 1).

Already before a complete understanding about the involved mechanisms can be gained, models constitute a useful tool to make a step forward in the awareness of soil-plant interactions and evaluation of the potential for C sequestration estimates under N deposition on the long term.
Presently, most of the soil models deal with soil N status control on C-cycle on short temporal scale or in N-limiting condition (Manzoni and Porporato 2008; Shibu et al.2006), proposing different formulations to model the interactions between the C and N cycles, varying in spatial and temporal scales, parametrization and data needed. In the case of increasing N availability, dynamical and statistical models deal mostly on the N effect on plant and woody biomass production (Parton et al.1993, Mäkipää et al.1998; Salih et al.2005; Pepper et al.2005) rather than on the effects within the soil inducing SOM accumulation due to the decline of microbial activity. In these models, SOM decomposition rate is affected by abiotic factors such as temperature, soil moisture and texture, while the strong dependence on N status is usually neglected or not well implemented.
To our knowledge, only few models trying to include the underlined mechanisms exist: the work proposed by Franklyn (2003) based on the continuous-quality decomposition theory and the dynamic models IBIS (Liu et al.2005) and SOMM (Chertov and Komarov, 1997). However on the one hand there is a need to keep such models highly dynamic , maintaining the biochemical information when proceeding to upscaling exercises, on the other hand, it is important to make the

most judicious mathematical simplification to make them manageable in the frame of predictive exercises.

The present work is a first attempt to model at small scale soil C and N interaction during litter decomposition and OM accumulation in response to an increase of internal and external soil N availability and hence the feedbacks in terms of C sequestration. The focus of our attention is the role of microbial community considered as a living component and not only as a simple soil pool (Fang et al.2005) underpinning the several feedbacks between C and N cycle under a biochemical point of view. The aim is to propose a semi-empirical formulation for investigating the N surplus control on the pattern of organic matter decomposition and accumulation starting from litter degradation, finding hence a trade off between the mathematical simplifications of the real process that current soil model offer and the mechanistic reasons behind the experimental soil responses observed.

3.2 Materials and methods

In order to ensure an extensive applicability of the model and to minimize the number of parameters to optimize, we decided to start from the soil C-scheme presented in the Century model (Parton et al.1987, 1993; Kirschbaum et al.2002). The step forward was to modify the N cycle in order to consider all the feedbacks between C and N dynamics.

3.2.1 Model description

Soil-C scheme

The basic scheme of soil C dynamic shares a lot of features with the century model that has been the *de facto* soil model in the literature for 20 years (Parton et al. 1987, 1988, 1993). In this way, we maintain a general scheme which is well known in the literature and widely validated and applied at different spatial and temporal scales (Schimel et al.1994; Kirschbaum et al. 2002; Li et al.2006). The model works on conceptual C-pools characterized by different capability to be degraded by soil microorganisms. The mathematical formulation follows a first order kinetic where the decomposition rates depend on several biochemical and physical factors that act multiplicatively to reduce the maximum potential organic matter degradation speed (Parton et al. 1987) (Table.1). Besides, the other fundamental hypothesis is the homeostasis of soil microorganisms with C-substrates and nutrients at each time step of observation, thus that to a unit of substrates decomposed corresponds a microbial growth without intermediate step. The soil C scheme is reported in fig. 1, the arrows indicate the fluxes of C in both organic and mineral form, e is the microbial efficiency, the parameter e_b constitutes the microbial metabolism cost, while β and γ

define the aliquots of C flux toward slow and passive pools; α are the reduction factors applied to decomposition rates.

Metabolic pool (MET) includes labile C compounds from litter and is extended to include inputs from rhizodepositions and low molecular weight compounds. The structural pool includes all the cellulolytic compounds (cellulose, holocellulose and hemicellulose), thereinafter simply referred to as cellulose pool (CEL). If it is not determined in laboratory (i.e. metabolic pool is chemically similar to water extractable carbon), labile pool is the fraction left in the litter after structural fraction determination. In case specific chemical information about litter is not available, the characterization of the main litter fractions can be determined following Kirschbaum (2002) once the lignin (LIG) and nitrogen content are known.

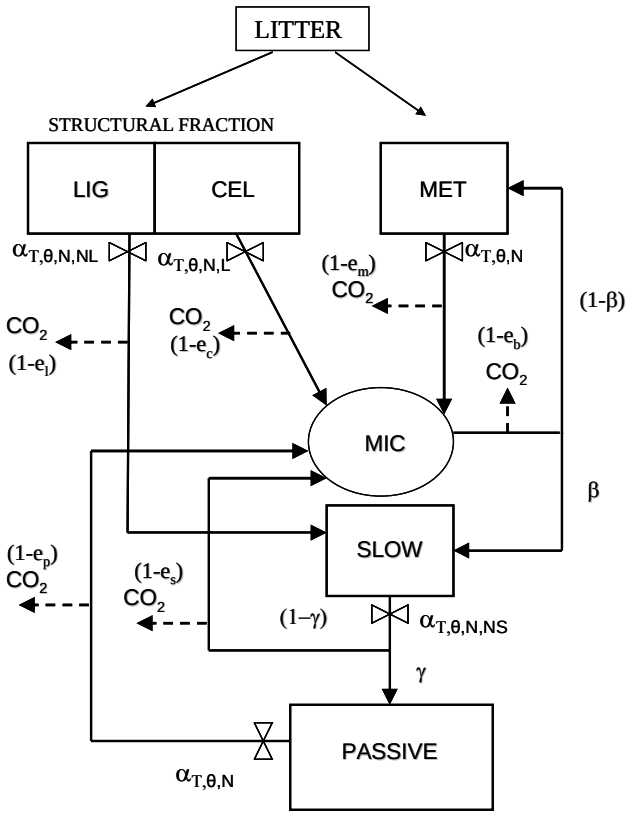

Fig. 1 Soil C-scheme, with indication of the C fluxes in both organic and mineral form; the C-fluxes, the decomposition of which is affected by N limitation or surplus are indicated. See text for more explanations.

When using the expressions soil pool or young pool, we will refer to the humic pool (conceptually indicative of slow C-pool with a medium turnover) where the interference of organic matter with mineral matrix is minimal. A passive pool follows as in the original scheme of Century model, where physical and chemical protection mechanisms occur (Six et al.2002).

The microbial pool (MIC) include all the microbial community of the soil neglecting all distinctions between species. The flux from the biomass pool includes microbial death cells (i.e. microorganisms death) and secondary products (Flaig 1971), while it is not the case in Century. This flux is split in two parts: the first is composed by the labile part of the microbial cell and enters directly the metabolic pool again, while the other part is incorporated in the microbial recalcitrant part (Van Veen et al.1984) and other resistant microbial by-products.

The microbial potential C uptake C_u is defined by the decomposition of C compounds, indicated by the fluxes D, affected by several abiotic constraints, including drivers of climatic nature, as temperature and soil moisture, constraints intrinsic of the structural part of the litter and due to mineral N content (table 1). The climatic inhibition factors, α_T α_θ are expressed following Kirchsbaum (2002).

$$Cu = \sum_{i=1}^{n} D_i e_i = \sum_{i=1}^{n} \alpha_T \alpha_\vartheta \alpha_{(N,Nj)} \alpha_L e_i K_i C_i$$

i=1, n; where n is the number of carbon pools, differentiating on the basis of their chemical recalcitrance. The higher the index, the lower the quality. In particular 1 is the metabolic pool. For simplification sake, we refer to m= metabolic pool; c=cellulolytic compounds; L= lignin pool; s=slow pool; p=passive pool. D is the decomposition C-flux; K is the potential decomposition rate, the subscript N is indicative of the N control on C decomposition; j=L when it applies to the lignin pool, S when the reduction factor is applied to the slow pool.

REDUCTION FACTORS	
αT	reduction factor due to soil temperature
αθ	reduction factor due to soil moisture
αN	reduction factor due to Nin limitation
αNL	reduction factor due to Nin excess applied to lignin
αNS	reduction factor due to Nin excess applied to soil
αL	reduction factor of decomposition of holocellulose encrusted in lignin matrix

Table 1 Description of the reduction factors affecting the different C-fluxes in the soil model.

The reduction factor α_L takes into account the encrustment of cellulose compounds in the structural fraction due to the presence of lignin and is expressed as reported in Parton (1987) and Henriksen and Breland (1999). Thus, the lignocellulosic index considering the control of lignin on celluloses decomposition (LCI), is defined as $C_L/(C_L+C_C)$.

parameters	**DESCRIPTION**
b_L	parameter included in α_L
b_{L2}	parameter included in α_{NS} e α_{NL} factor
λ	microbial death rate and release rate of microbial by-product
K_m	decomposition rate for metabolic pool
K_c	decomposition rate for cellulosic pool
K_l	decomposition rate for lignin pool
K_s	decomposition rate for the slow pool (humic substances, young C pool)
K_p	decomposition rate for the passive pool (protected C fraction, old C-pool)
e_m	microbial efficiency on metabolic pool
e_c	microbial efficiency on cellulosic pool
e_l	fraction of C of lignin incorporated in slow pool
e_s	microbial efficiency on slow pool
e_p	microbial efficiency on passive pool
e_b	fraction of C respired during microbial turnover
β	fraction of organic matter that enters the slow pool
γ	fraction of organic matter that enters the passive pool
k_{bu}	microbial uptake rate of mineral N
CN_{bmax}	max C/N for microbial pool (newly formed microorganisms)
CN_{bmin}	min C/N for microbial pool (newly formed microorganisms)

Table 2 Description of the main parameters of the soil model.

Soil-N scheme

The N content of the litter in the different constituents where determined following the scheme proposed in Kirschbaum (2002). Although strictly speaking, N is not included in the chemical composition of lignin, as a first attempt we assume that N content in the structural pool is equally distributed in lignin and cellulose pools, considering that some N becomes quickly trapped within the lignin matrix. The N fluxes from the C pools are proportional to their respective C fluxes knowing the C to N ratio of the source pools. We consider that the N of the metabolic pool can be assimilated by microbes directly in organic form as simple molecules (i.e. small amino-acids), once metabolic pool is degraded in low-molecular weight compounds by exocellular enzymes. These substrates can be thereinafter used directly for building microbial tissues or proteins or deaminated internally (Barraclough 1997; Schimel and Bennet 2004; Roberts et al.2009). The excess of N

behind the microbial N need is mineralized internally and expulsed outside the microbial cell. Actually the hypothesis behind the proposed scheme correspond to a higher affinity of

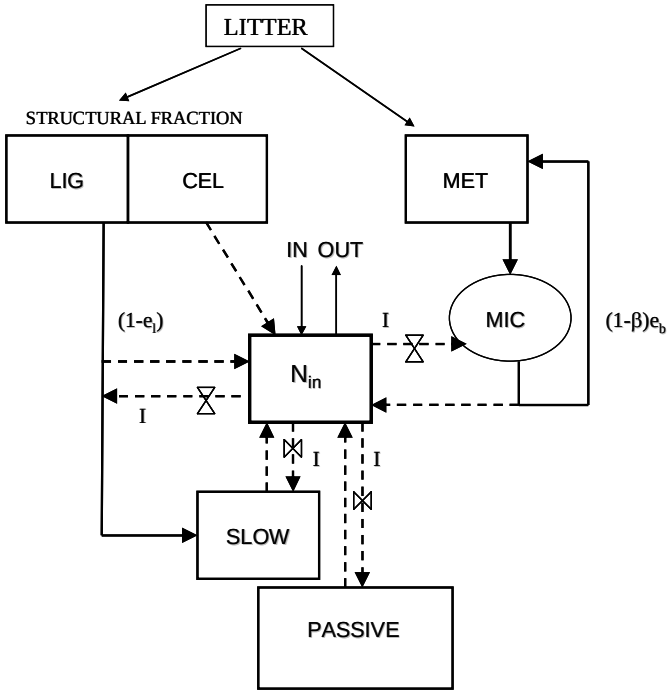

Fig. 2 Soil N-scheme. Bold line indicate organic N flux while dotted line mineral N fluxes. I items constitute the immobilization fluxes, IN constituted all the external N input as fertilization and N depositions, OUT constitutes all the gaseous losses, leaching and plant uptake.

microbes with organic N than with mineral N, taken up when organic N is not enough to cover the stoichiometrical need. N in the other C-pools are directly mineralized increasing mineral N pool, from which N follows different pathways including uptake by microorganisms, abiotic immobilization, plant uptake, leaching and gaseous loss. The N model includes thus both the MIT and DIR scheme (Hadas et al. 1992; Barraclough 1997), with MIT scheme working at level of fresh and C-rich substrates while MIT at level of SOM, where usually a net N mineralization is observed. Analogously for C uptake, the potential N uptake is defined as the sum of organic and inorganic uptake (see table 4 for parameter values):

$$Nu = \frac{K_m C_m \alpha_T \alpha_\vartheta \alpha_N}{CN_m} + K_{bu} N_{in}$$

where CN_m is the C/N ratio of metabolic pool, K_{bu} is the rate of microbial N uptake and Nin is the mineral N pool as reported in fig. 2. The second term on the right hand side of the equation is the biotic immobilization and is assumed to be directly proportional to the mineral N pool.

Abiotic immobilization of mineral N contributes to retain N in the SOM and include condensation of inorganic N on phenolic compounds during the humification process (Magill and Aber 1998) or enzyme-like rather than chemical reactions (Davidson et al.2003). For these reasons, we maintained the same formulation as proposed in Century where the immobilization of mineral N is regulated by the C/N ratio of the newly formed organic matter; It may evolve between the values fixed in Parton (1993), depending on a critical value of inorganic N of 2 g N m^{-2}. The immobilization of mineral N interests all the C fluxes toward slow and passive pools, including the flux from the lignin pool. Concerning the latter it was assumed that the minimum C/N ratio of newly formed organic matter is equal to the C/N ratio used for the other C fluxes.

The other N outputs from the system can be computed as proportional to the mineral N pool. Once the sum of all the loss is larger than the total soil mineral N availability, the priority is given to microbial and plant uptake and abiotic immobilization. Thereinafter the other losses (i.e. leaching and gaseous loss) are computed again on the updated Nin pool.

3.2.2 C-N interaction on decomposition processes

Several soil models deal with C-N interactions on decomposition of fresh and C-rich substrates depending mostly on C to N ratio of compounds and N demand of microbes, defining in different way how the pathways of C and N are regulated when one of the two elements is limiting the microbial anabolic functions. In particular the excess of the non-limiting element is considered to be mineralized as CO_2 or mineral N, following other anabolic pathways (Manzoni and Porporato 2008; Hadas et al.1998; Molina et al. 1983). In the present scheme, when new microbial biomass is built, the ratio between C uptake and total N uptake determines the C to N ratio of the new tissue. It may span the range between 6 and 12, and is defines if the systems is N limited, when $CN_b > CN_{bmax}$ or if a surplus occurs, when $CN_b < CN_{bmin}$. In both cases, a reduction of carbon degradation is considered, as a response to a reduction of the microbial growth rate.

In the first case, C-excess again mineral N, there is a decrease of microbial activity and particularly C-substrates decomposition according to Molina (1983) until the CN_{bmax} of newly microbial biomass is satisfied. The reduction factor is applied to every C flux of decomposition, including the one corresponding to the decomposition of the metabolic pool.

$$\alpha_N = \frac{K_{bu} N_{in}}{Cu / CN_{b\max} - D_m / CN_M}$$

Once the inorganic N increases in the soil solution, the inhibitory effect of N, that controls both directly and indirectly the microbial activity, is expressed again as a lower decomposition rate and hence a lower microbial growth rate. At the same time reducing decomposition rates mimic a decrease of C-substrates quality. Concurrently the C/N of newly formed microbial biomass moves toward a low value, i.e.6, leading to a decline of the average C/N ratio of the whole microbial community, simulating a shift toward a system with a more prononce domination by bacteria.

The real type of inhibitory effect of mineral N on soil processes is still not clear; Several mechanisms have been proposed, both of chemical and biological nature, but the relative weight of them, their magnitude and even direction have still to be determined. We supposed that the presence of mineral N has a direct and an indirect effect on microbial community functions. More precisely, we assumed that the reduction factor may be expressed as an explicit function of inorganic N availability considering the microbial N_{in} demand and N_{in} availability at a specific time step. This choice is based on the hypothese that an equilibrium between microbes and N status in the surroundings has been achieved:

$$F = \frac{N_{availability}}{N_{demand}}$$

$$\alpha_{NS,L} = \exp(-b_{L2} LCI(F-1))$$

b_{L2} is the parameter to be estimate. The same relationship holds for the reduction factors α_{SL}, for soil compartment (see table 1).

In this framework, the mechanism leading to the decomposition process is not directly dependent on the absolute rate of N deposition nor on other inputs or outputs, but is a function of the availability of mineral N that can be automatically generated by the model. Compared to the model presented by Chertov and Komarov (1997) the proposed formulation allows the mineralization process to increase again once the mineral N content decreases, thus mimicking that initial decreased SOM decomposition is not a persistent phenomenon when the external disturbance stops. Moreover in the SOMM model (Chertov and Komarov, 1997) the reduction factor was function of organic matter content, focusing hence mainly on the N-effect on organic matter recalcitrance rather than a direct effect on microbial community.

The type of ecosystem and vegetation is indirectly considered in the LCI index, through the the information on the amount of lignin in the litter and through the parameter b_{L2}. This simple mathematical formulation states that the inhibiting effect of mineral N is more evident when LCI is higher; hence N has a higher inhibitory impact for forests than for grassland ecosystems and on old litter than fresh litter. The explanation behind these statements is related to the assumption that N has a more significant effect on fungal dominated ecosystems, thus on soil dominated by low

quality litter (Sinsabaugh et al.2002; Blackwood et al.2007; Berg and Matzner 1997) or where lignin is the most significant components of structural litter, thus affecting the oxidative activity. Initially fungi have been regarded as much more sensitive than other microbial species to increased mineral N. But some recent field experiments have underlined that N could affect soils with a bigger share of bacteria (Gallo et al. 2004; De Forest et al.2004; Blackwood et al.2007), suggesting that this dynamics could be a more ubiquitous phenomenon than previously endorsed. Thus, we assume that a chemical effect is reached even in ecosystems with weakly lignified litter due to acidification process. Hence the LCI index impacting the reduction factor takes into account this limitation, but whit a lower inhibiting power than shown in fig.3, A-B.

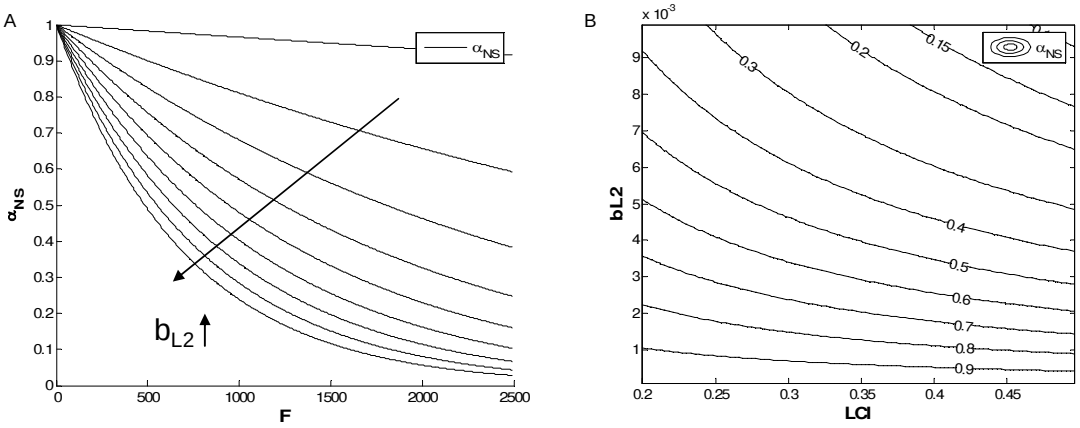

Fig. 3 A) Behaviour of the reduction factor as function of parameter bl2 and LCI=0.35; B) behaviour of the reduction factor as function of bl2 and LCI for a fixed values of F=500.

This parameter b_{L2} makes the reduction factor highly sensitive to significant amount of mineral N, while the response is similar among litter quality and ecosystems for lower amount of mineral N.

As a matter of speculation, we assume that the microbial community growing on the more recalcitrant part of litter is similar to the population growing on soil organic matter with regard to the response to N addition, as suggested by Blackwood (2007) and that it is defined by a site-specific parameter b_{L2}. Actually, the same type of enzymatic response, i.e. lower oxidative activity, was observed for the litter and soil originated from the same plot (Sinsabaugh et al.2005). Hence we assumed that the relationship describing the negative influence of N is hold between the lignin and the soil pools. In particular the same function was applied to the young soil pool, neglecting any N control on the passive C-pool. In the latter, other physical and chemical effects may occur on the microbial activity and are likely to have a higher impact.

3.2.3 Model parameters

The estimation of parameters was conducted taking into account the aim of the work, ie the evaluation of the model performance and the investigation of its capability to reproduce the numerous soil experimental evidences under N fertilization. Secondly, the lack of mechanistic knowledge of the biochemical processes and the gaps of all the experimental data necessary for optimization purposes lead to the need to minimize the number of unknown or poorly constrained parameters. Thus some of the model values were chosen from the original Century model (Parton et al. 1987, 1993) and other chosen based on the information available in the literature, and determined either in laboratory experiment or in field experiments (table 4). Lastly, the parameters showing the highest between studies variability and significant sensitivity toward model output were optimized using a set of experimental data taken from the literature.

Decomposition parameters

In several soil models, decomposition constants are estimated from laboratory studies under controlled conditions or defined a priory as potential values and the size of soil C pool is determined using the equilibrium the system should reach under specific climatic conditions and litter input (Century, Roth-C). In the present work, focussing mainly on C and N interaction on the recalcitrant fraction, we decided to use average decomposition values independently from climatic condition. As a first approximation, rather than using decomposition data obtained on the single fraction degraded in laboratory incubation, we used the decomposition rates obtained from Minderman (1968) in long term field litterbag experiments in a Dutch forest site (average temperature of the site X °C). In that study, Minderman determined the degradation rates of different chemical litter constituents, in particular of the metabolic, holo-cellulosic and lignin ones (table 4). The decomposition rate Ks for the slow pool is described by a wide range of values in the literature due to the difficulties to find a correspondence between the conceptual pool and the chemical characterization of the real soil pool. For instance, the estimations of Ks with laboratory incubation studies are situated in the wide interval that separate the estimate of Hadas (1993), 0.0001 d-1, and the one of Nicolardot (1994), 0.006 d-1 at 28°C.

Microbial parameters

The C/N ratio of microbial community is allowed to fluctuate between 6 and 12 in order to mimic the presence of more or less bacteria compared to fungi (Dommergues and Mangenot, 1970) rather than luxury uptake of inorganic N under increased N availability. Microbial efficiency values were taken from the litterature and laboratory experiments where microbial growth on a specific

substrates was investigated (table 4). Although the difficulties to define an absolute value for the microbial growth yield on single C-compounds, we decided to maintain the values of Century. Hence,we assume a decreasing efficiency with increasing recalcitrance of substrates but a hither efficiency for low C/N substrates (Lekkerkerk et al. 1990, Chiellini et al. 2007).

Parameter λ constitutes the contribution of microbial mortality and release of by-products. Contrary to the microbial growth rate which is linked to the C-uptake and hence to organic substances decomposition, the definition of λ is more ambiguous due to the experimental challenge represented by the measure and the splitting of the two aliquots. In the literature, the equivalents to the parameter λ range between $0.95d^{-1}$ for Blagodastky and Richter (1998) who tried to compute the mortality rate, and $0.001d^{-1}$ as estimated by Mueller (1996). The values given by Cochran (1988) for the labile part - 0.2 d^{-1} - and Van Veen (1984) for the recalcitrant part of microbial tissue -, 0.005 d^{-1} - lay in-between.

Parameter K_{bu} is important particularly at the beginning of the decomposition of the fresh litter or for the growth on C-rich substrates where the uptake of mineral N covers the microbial need. Firstly, the estimation of this parameter would require a detailed set of data particularly on short temporal scales. As it is out of the aim of the present work; we kept the estimates of K_{bu} from field experiments reported in the litterature (Puri and Ashman, 1999; Chen and Stark, 2000; Micks et al.2004). However it is important to underline that only its order of magnitude ($0.1d^{-1}$) was estimated, due to the difficulty of the evaluation of the relative contribution of abiotic and biotic immobilized substances..

3.3 The model : sensitivity analysis, optimization, evaluation.

The model presented above has been used to analyse the litter degradation and organic matter accumulation following the pathway of the different soil fractions throughout time as reported in fig. 4:

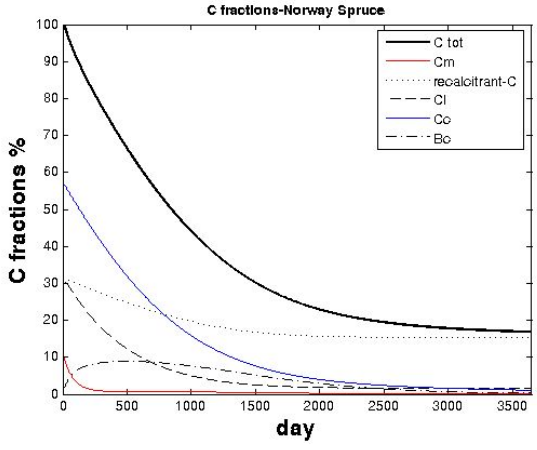

Fig. 4 Qualitative behaviour of decomposing Norway Spruce litter during 10 years.

K_s, λ, b_{L2} were the model parameters that have been optimized. As explained earlier on, this choice was driven by the large range of values reported in the literature and the sensitivity of the output to the variation of these parameters. Actually, we evaluated the sensibility of the output to changes of K_s, λ, b_{L2} ±50% in the frame of a decomposition experiment of pine litter which lasted almost 5 years under high rates of fertilization. We compared the model results to a case of reference where the parameters of interest gave an initial good qualitative fit to the data (organic N and recalcitrant C). As expected, the parameter λ greatly influenced the retention of organic N with a surplus of N of 50% in respect to the reference case in just 5 years, while the other parameters controlled the C dynamics (Fig. 5).

We fixed the a priori range within which the parameter Ks may fall during the optimisation: between 10^{-5} d^{-1} and 10^{-3} d^{-1}. The maximum was fixed based on the estimation of Minderman (1968) of the decomposition of phenols and lignin : $0.0019d^{-1}$.

The parameter b_{L2} was allowed to take any value between 10^{-4} and 10^{-2} (Fig. 3). Though the larger range of values present in the literature, the parameter λ was optimized keeping 10^{-3} and 10^{-2} as limits due to the higher sensitivity of the model output to this parameter..

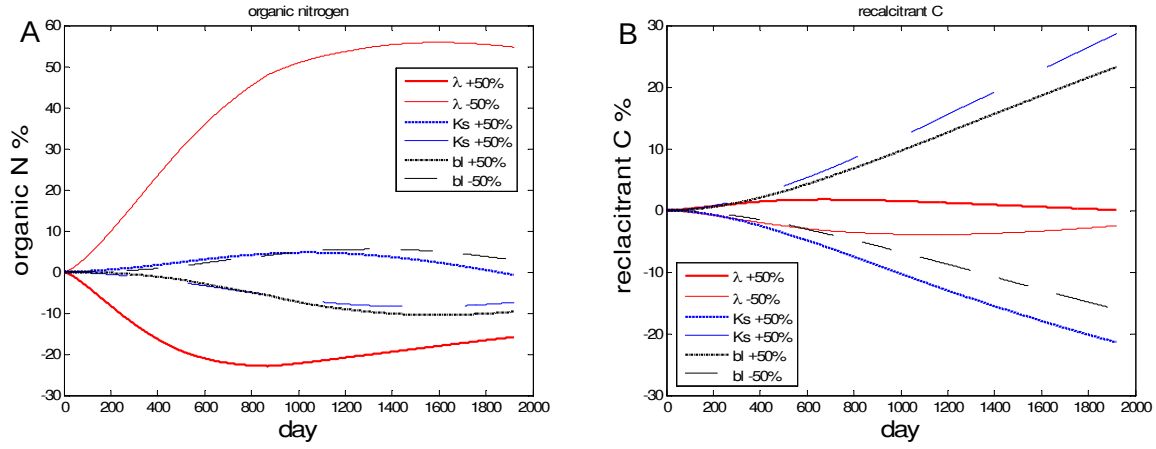

Fig. 5 Response of model output to a change of ±50% of the parameter values compared to a situation of reference. A) Organic nitrogen; B) recalcitrant carbon.

The parameter K_{bu} is an other sensitive factor which may play an important role on the model performances. However, as previously underlined, a series of high frequency data at short time scale would be necessary to constrain it in a rigorous way.

The dataset used for optimization and evaluation, belong to the litterbags experiment conducted at the Harvard Forest LTER under different fertilization rates during the period 1988-1994 (Magill and Aber 1998; Magill et al.1997). The experiment was carried out using different litter types of a

coniferous and hardwood stand. Mass loss, N, cellulose and lignin fractions were determined. In particular, we assumed that the estimated lignin fraction is equivalent to the total recalcitrant C in the litter bag (lignin and humified substances) due to difficulties to discriminate lignin compared to others soil phenolic polymers. The experiment documented a high mass lossaccross sites and litters, with a reduction ranging from 12.3 to 21.7 % on average with increasing N supply. The total extractable mineral N in the forest floor measured during field campaign was used as a proxy of inorganic N availability (Magill et al. 1997). As previously mentioned, in the present work, the model evaluation phase is performed without knowing the site climatic condition during the experiment. Hence the decomposition constants of Minderman (1968) were used, while the ability of the model to reproduce patterns among different types of litter and to reproduce C and N sequestration was evaluated.

From the whole set of the litterbag study, one subset was extracted for the optimization of the three parameters λ, Ks and b_{L2}. In particular we decided to fit the model to litter bag data under the highest N treatment, since this condition showed the maximum inhibition of organic matter decomposition and allowed the most satisfying discrimination of the parameters accross the two different sites, covered by conifers and hardwood species. A second optimization procedure was performed keeping fixed K_s, λ and fitting the site-specific parameter b_{L2} separately, using pine data on the pine stand, and oak litter on hardwood stand. It was assumed that b_{L2} depends on the specific microbial community that has developped under a particular ecosystems and a particular aboveground tree species composition (DeSanto et al.2002). It takes into account the colonization of the autochtonous microbes on the new litter. After the sensitivity analysis (Fig.5), we used only C data in order to optimize the b_{L2} value, for which C values were more discriminant than the one of organic N.

The initial condition for the simulation was fixed at the ninth month of the litter-bags experiment in order to avoid the issues that could be associated with an overfit which would correspond to the reproduction of evolution associated with processes occurring on short temporal scale that we did not represented; Dissolved organic C production and leaching of labile C, like other mechanisms that may cause a rapid change in litter chemistry are difficult to model because of the paucity of experimental data. Starting from the chemical characterization of mass remaining at the ninth month, we reconstructed all the C and N pools of litter-bag mass following Kirschbaum (2002). C represents 50% of the mass at the beginning. A reasonable guess, assuming that the initial microbial-C pool was equal to 1% of the total C pool was made. Initial average C to N ratio within the microbial community was fixed to 8 as a first attempt . We kept the slow pool equal to zero, as there is no interaction between humic substances and the mineral matrix during a litter-bag study.

After that optimization was performed on one subset of data, the other was used to evaluate the model predictive ability. Different litter types and N treatment (including the ambient condition) were considered in reference to different combination of C-compounds and soil N availability. The performance of the model in reproducing the remaining mass, recalcitrant pool that includes both lignin and humic substances and C/N of litter bag throughout the fertilization experiment were tested. The aim was to evaluate the capacity of the model to reproduce, the main pattern of C and N retention considering the feedbacks between the two cycles under increasing mineral N supply without taking into account the climatic conditions. In particular, the capacity to model SOM formation and C/N ratio at each point was used to test the assumption behind the model formulations.

3.4 Qualitative study

A qualitative/quantitative study was performed in order to investigate the modelled behaviour of decomposing litter and SOM accumulation under different N status. We simulated with the optimized model the sensitivity of the C retention when considering an increase of external N content (mineral N) and internal N content of litter (organic N) for different types of litter. Considering different initial amount of N, within the litter allows to deal with the difference of characteristics between broadleaves and conifers or with the indirect effect of climate associated with shift of species or the change of N content of leaves due to N deposition. In this a first attempt, changes in lignin and N content directly linked to climatic patterns were not considered, since no specific relationships between the three.

Several litter type and chemical characteristics have been collected in studies available in the literature. Our goal was to use realistic combination of N content and C-fractions measured analytically in laboratory rather than the one following Kirschbaum (2002). Litter data corresponded to several sites among temperate European and American forests, including some Mediterranean sites, and exhibited a wide range of N concentration, although most of the samples showed values between 0.3-1.15 % N (Table 3). *Populus tremula* litter was included because of its high N concentration : 2.42 % N.

Some grass litter data were also included in our list in order to investigate qualitatively the behaviour in a non forest ecosystem type.

We conducted a decomposition study on a 100 g C sample of a specific type of litter. A reference depth of 10 cm was used and decomposition was performed and stopped once the decomposition rate had decreased below 0.005 % d^{-1} in order to simulate the time when the mass loss is

sufficiently small to consider that we have a good estimate of the final conditions. The value of the latter against the initial ones with regards to external and internal N content and litter quality were considered.

In the first simulation, a fixed amount of 0.2, 0.5, 0.9 and 1.5 g N m^{-2} was employed to check the sensitivity of the suppression of degradation, keeping the soil mineral N constant along incubation and affecting thus decomposition since the beginning. This could simulate a persistent input from N deposition or an increase of mineral N due to diffusion from other soil layers after increasing SOM mineralization due to higher temperature. In the first case, the effect of internal N is indirectly included as well, since it regulates the microbial demand of external mineral N.

In the second experiment, the same level of initial background level of 0.5 g N m^{-2} was chosen and mineral N was allowed to accumulate in the system after the occurrence of a net mineralization, without any N loss that follows litter decomposition and SOM accumulation depending on internal N. In order to make the two results comparable , the simulation were performed with the same decomposition rates as used in the evaluation section. For instance, the information about the climatic conditions have been neglected again. Actually, the abiotic constraints operate multiplicatively when reducing decomposition constants, and have the same control on all the C-pools fluxes. Thus we expected only a change of the remaining mass as a function of N status, while the qualitative behaviour would not change.

LITTER TYPE	N	Cm	Cc	Cl	L/N	REF.	SITE
	%	%	%	%			
Red Pine *(Pinus Resinosa)*	0.64	36.0	38.6	25.5	39.8	Magill and Aber 1998	Mitchigan, U.S.
Scots Pine (*Pinus sylvestris)*	0.36	12.2	60.1	27.7	76.9	Berg et al.2003	Sweden
Silver Fir *(Abies alba)*	1.3	25.9	31.4	42.7	32.8	Berg and Meentmeyer 2002	Sweden
Norway Spruce *(Picea abies)*	0.77	10.9	57.4	31.7	41.2	Berg and Meentmeyer 2002	Norway
lodgepole pine (*Pinus Contorta)*	0.31	14.3	48.1	37.6	121.3	Berg et al.2003	Central Sweden
Ponderosa Pine *(Pinus Ponderosa)*	0.77	21.4	47.7	30.9	40.1	Hart et al.1992	California
Stone Pine *(Pinus Pinea)*	0.3	20.5	48.1	31.2	104.0	Berg et al.2003	south Italy
Iron tree (a) (*Metrosideros)*	0.23	19.4	69.0	11.6	50.4	Hobbie 2000	Hawaii
Iron tree (b) (*Metrosideros)*	0.31	41.0	48.5	10.5	33.9	Hobbie 2000	Hawaii
Trembling Aspen (*Populus tremula)*	2.42	31.9	45.1	23.0	9.5	Berg et al.2003	central Sweden
White birch (*Betula pendula)*	0.95	30.4	40.8	28.8	30.3	Berg and Mentmeyer 2002	Sweden
Cork oak (*Quercus suber)*	0.81	44.8	37.1	18.1	22.3	Gallardo and Merino 1993	south Spain
White oak (*Quercus Alba)*	0.84	32.4	47.4	20.2	24.0	Aber et al.1990	Wisconsin and Massachusetts, U.S.
Holm oak (*Quercus ilex)*	0.88	13.4	62.4	24.2	27.5	Cortez et al.1996	Southern France
Black birch (*Betula lenta L.*)	1.15	34.0	43.6	22.4	19.5	Magill and Aber 2000	New England, U.S.
Black oak (*Quercus velutina*)	0.76	37.1	38.6	24.3	32.0	Magill and Aber 2000	New England, U.S.
Turkey oak *(Quercus Cerris)*	0.76	39.8	33.8	26.4	34.7	Garhooee 1998	Tuscany, Italy
Downy oak *(Quercus Pubescens)*	0.84	56.4	25.1	18.5	22.0	Garhooee 1998	Tuscany, Italy
Grass, mixed	1.14	30.0	58.0	12.0	10.5	Taylor et al.1991	Rocky Mountains, U.S.
Wheat grass (*Triticum aestivum)*	1.44	35.0	59.0	6.0	4.2	Lynch 1987	/
grass *(Festuca)*	0.95	63.1	20.9	16	16.84	Zourkoura et al.2003	Greece

Tab. 3. C and N constituents of different type of litter used in the model experiment. N, nitrogen content; Cm, metabolic C fraction; Cc, cellulosic C fraction; Cl, lignin C fraction; L/N, lignin to nitrogen ratio.

3.5 Statistical analyses

Model parameters optimization was performed using a nonlinear least square fitting algorithm that allows to constrain the range of values for the chosen parameters, as indicated in the previous section (preconditioned conjugated gradient optimization method; Matlab software 2004, version 7.0.1, R14SP1). Since experimental data presented different order of magnitude, data were homogenized dividing the values by their initial standard error. However, since the error associated to the experimental measures was missing, it was not possible to evaluate an error associated to the parameter obtained. A model evaluation was thus carried out in order to test the robustness of the working hypothesis and the formulation proposed. The performance of the model was assessed using regression methods by verifying the hypothesis on the intercept and the slope of the model outputs against observed variables. Moreover other statistical indexes have been calculated; the index U, that is the RMSE divided by the sum of RMSO observations and RMSP prediction, including hence different component of bias, ranging between 0 for the good fit and 1; the model efficiency (EF), ranging between -∞ to 1; the consistent over- or under-prediction errors of the models (bias errors MD) were evaluated by calculating the mean difference between the simulated and the observed data (Smith and Kenneth 1995; Smith et al., 1997; Smith et al.1996).
The statistics were elaborated with STATISTICA version 7.0 (data analysis software, Stat.Soft. INC.2004).

$$U = \frac{\sqrt{\frac{1}{N}\sum (O_i - P_i)^2}}{\sqrt{\frac{1}{N}\sum O_i^2} + \sqrt{\frac{1}{N}\sum P_i^2}}$$

$$EF = \frac{\sum_{i=1}^{n} (Oi. - \overline{O})^2 - \sum_{i=1}^{n} (Pi - Oi)^2}{\sum_{i=1}^{n} (Oi - \overline{O})^2}$$

$$MD = \frac{1}{n}\sum_{i=1}^{n} (Pi - Oi)$$

3.6 Results

3.6.1 Optimization of parameters and evaluation

Optimized parameters and the model constants are reported in table 4. Comparison between experimental and modelled data is shown in figure 6,A-F while statistics are reported in table 5,6. Results have been presented considering the N treatment without statistical elaboration of data on each site separately.

parameters	**value**	**unit**	**bib. ref.**
b_L	3		Parton et al. 1987
b_{L2}	0.0023,0.0032		Coniferous stand, hardwood stand
λ	0.0028	d^{-1}	see text
K_m	0.012	d^{-1}	Minderman et al.1968
K_c	0.003	d^{-1}	Minderman et al.1968
K_l	0.0019	d^{-1}	Minderman et al.1968
K_s	0.0007	d^{-1}	see text
K_p	$2.7*10^{-6}$	d^{-1}	Parton et al.1987
e_m	0.5		see text
e_c	0.4		Van Veen et al.1985, Chiellini et al.2007
e_l	0.7		Parton et al.1987, Van Veen et al.1985
e_s	0.45		Parton et al.1987
e_p	0.45		Parton et al.1988
e_b	0.5		fixed a priori
β	0.3		Molina et al.1983
γ	0.03		Parton et al.1987
k_{bu}	0.1	d^{-1}	see text
CN_{bmax}	12		see text
CN_{bmin}	6		see text

Tab. 4 Parameter values in the decomposition model from literature and estimated from optimization procedure. …….

The optimized parameter b_{L2} is 0.0023 for pine stand and 0.0032 for Hardwood stand. However, when the model is applied for low values of mineral N, the discrimination between stand types is weaker.

Computed mass loss data were lower in the high N treatment, in particular in the hardwood stand (Fig. 6 C), due to difficulties to model cellulose degradation, the decomposition of it is tightly linked to decomposition of lignin in a non linear way (Sinsabaugh and Moorheard 2006), modulating the cellulose exposition to microbial attack.

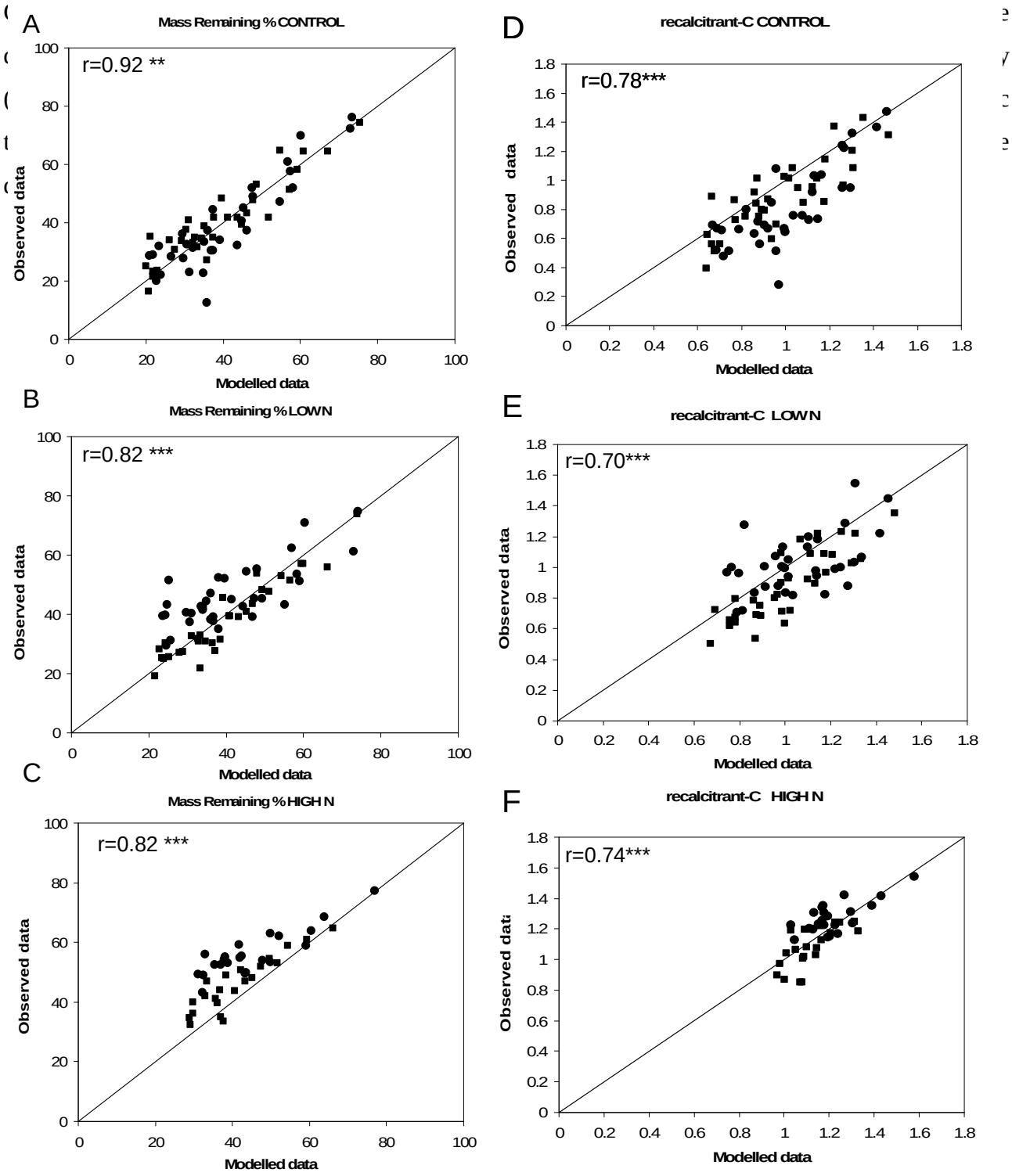

Fig 6 Measured against modeled mass remaining in the litterbags expressed as percentage in the A) plot control, B) low N treatment, C) high N treatment. Measured against modeled recalcitrant-C expressed as g C per litter bag in the D) plot control, E) low N treatment, F) high N treatment. The line indicates the 1:1 relation. Square symbols refer to litter on pine stand, circle symbols refer to hardwood stand. Correlation coefficients are reported with ** P<0.01; *** P<0.001.

Experimental data are the results of the average of several litterbags. As most of the attention has been given to organic C and N retention, discrepancies between observed and predicted C/N values at the early stage of decomposition (data not shown) may be explained by the missing optimization of K_{bu} rather than λ at short temporal scale. This is due to the paucity of observations during the first year of field campaign. This is especially the case for the litter chemistry and mineral N in to the soil.

Although the optimization of b_{L2} has been conducted using only pine litter data for the pine stand and black oak litter for the hardwood stand, the model is able to reproduce the behaviour of litter decomposition depending on its quality. In particular, the best performances are obtained on the pine stand, while the results were not satisfying for the hardwood stand under high N treatment (this may be due to a poor representation of the cellulose degradation). Other test have been carried out considering fixed C/N ratio of microbial biomass equal to 8 but with less satisfactory results in particular for organic N content (data not shown) and hence the capability to simulate the dynamic of organic N retention in the organic matter.

t-test mean values				t-test intercept			
α=0.05	P-value			t-stat=1.99	t-value		
TREAT.	A	LOW N	HIGH N		A	LOW N	HIGH N
MR	P=0.99	P=0.44	P<0.01	MR	0.19	0.62	1.01
rec-C	P<0.01	P<0.01	P=0.83	rec-C	-1.94	9.47	5.82
CN	P=0.30	P=0.64	P=0.65	CN	-1.03	-0.38	-0.126

Tab. 5 Statistics for the model performance evaluation. MR, mass remaining; rec-C, recalcitrant C, CN, mass C/N ratio. Level of significance α=0.05.

	U	**EF**	**MD**
MR A	0.06	0.80[a]	0.019
MR LN	0.08	-0.18	-1.71
MR HN	0.09[*]	-0.09	-7.88[*]
Rec-C A	0.1	0.33	0.13
Rec-C LN	0.08	0.03	0.08
Rec-C HN	0.04	0.055	-0.006
CN A	0.10	0.94	-1.52
CN LN	0.09	0.95	0.35
CN HN	0.11[a]	0.90	0.31

Tab. 6 Statistics describing the performance of soil model predictions. MR, mass remaining; Rec-C, recalcitrant carbon; CN, mass C/N ratio; A, ambient condition; LN, low nitrogen treatment; HN, high nitrogen treatment.

Intercept and slope test were performed in order to evaluated the modelling power. The hypothesis test of null-intercept on the regression line between modelled and observed data was computed, showing that the null-hypothesis (no bias) could not be rejected for most of the variables (P=0.05; t-stat=1.99). Only the recalcitrant-C modelled exhibited a bias in the low and high N treatment, although evaluating the mean differences in the latter case, recalcitrant-C showed a good agreement between the model and experimental data (Table 5).

The statistical test on the unitary slope of this regression line was computed as well. Student two-side t-test failed to reject the null hypothesis at significance level 0.05 in some of the variables considered. Yet the hypothesis of no mean difference had to be rejected in the case of the mass remaining in the high N treatments and in the case of recalcitrant-C in the control treatment (Table 5). Predicted and observed C/N generally agree quite well across sites and litter type, meaning that although the bias in determining recalcitrant-C accumulation, the modelled mechanisms behind C and N incorporation in SOM are robust.

3.6.2 Qualitative study

The qualitative study was performed using chemical data of litter coming from different natural ecosystems; data were clustered depending on the plant function type - coniferous, broadleaves, grassland - and on the climatic zone i.e. temperate, boreal or mediterranean.

EXTERNAL N

The model experiment was conducted with different constant background levels of mineral N during the decomposition dynamic and let the model run in order to see the sensitivity of the final remaining C to different litter qualities, in particular the quality of C-compounds, i.e. of the structural fraction. As expected the signal was higher for high-lignin litter (Fig. 7), but the response in terms of C accumulation increased for higher values of mineral N, while it remained comparable among litter type for low N availability. Model results show how broadleaves may accumulate more C compared to conifers, because broadleaves litter contains more N. After the mechanism proposed in the present work, the higher N content of broadleaves affects in turn the balance between microbial mineral N demand and N external N availability modulating repression mechanisms.

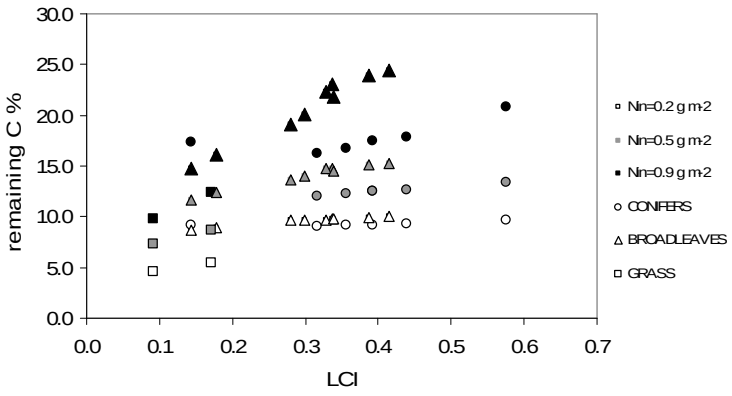

Fig 7 Remaining C for different type of litters as function of initial litter LCI and for three different constant level of mineral N.

In conditions of higher N content the mass remaining in the system increased and the C/N ratio of recalcitrant-C declined, thus the organic fraction of young carbon showed a higher percentage of N as it was expected by the model (Fig. 8). Data were averaged and clustered by plant-functional type. The recalcitrant-C reported includes all the compounds of phenolic origin (lignin), in their original form rather that chemically transformed in lignin-like substances and humus. Therefore, the correspondent transformed N does not include labile N forms such as metabolic compounds and microbial N. The accumulation of C is due to the decrease of lignin and to the decomposition of newly formed humus substances balanced by the decreased or increased cellulose degradation, since cellulose degradation is not limited by N. For instance it is important to keep in mind that the net C-sequestration is always the balance between the decomposition of different pools, that exhibit different response to mineral N. Finally the fig. 8 showed that broadleaves and conifers exhibited similar recalcitrant C/N ratio over a broad range of fractions of recalcitrant C while grass litter exhibited a different behaviour.

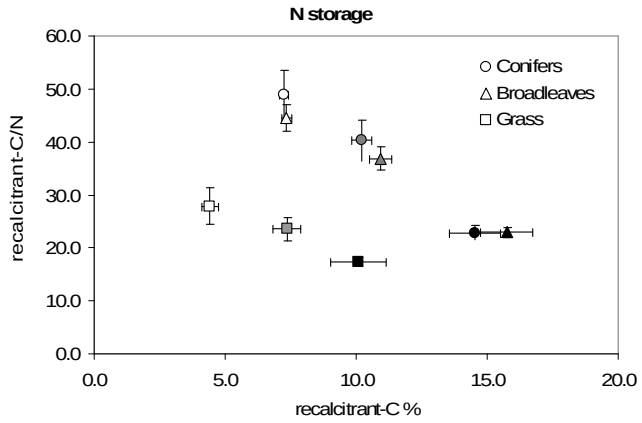

Fig 8 Remaining recalcitrant-C for different type of litters versus the C to N ratio of the remaining recalcitrant C and for three different level of mineral N, white N=0.2 g m^{-2}; grey N=0.5 g m^{-2}; black N=0.9 g m^{-2}. For a clearer representation, data have been clustered as plant functional type.

When plotting decomposed C as percentage % of the initial mass-C versus external N g^{-1} litter, a linear pattern was observed (Fig.9). The dispersion of data around the average slope is due to the importance of litter quality, initial concentration of lignin and N, that makes more sensitive litter decomposition rates under different level of external mineral N.

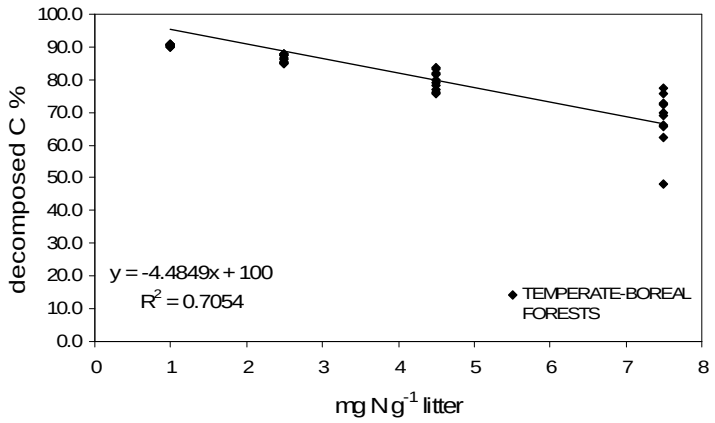

Fig 9 Decomposed C as % reported versus external litter N concentration expressed as mg mineral N g-1 litter. Data represent only litter data from broadleaves and conifers of the temperate areal. Values are computed for different soil inorganic N value as background equivalent to 1, 2.5, 4.5, 7.5 mg N g-1 litter.

When including the results obtained for temperate-boreal forest litters, a potential accumulation of 4.5 % C per 1 mg external N g^{-1} litter mass has been estimated. Actually this value constitutes a potential limit since N losses or plant interaction have not been considered in the simulation and the model-run was stop before the decomposition rate effectively decreased to zero. Moreover in natural ecosystems, other factors following humification process are important such as the stabilization of young carbon in the mineral matrix, which may contribute to the immobilization of C substrates over millennia.

INTERNAL N

Analogously to the previous qualitative study, we reported the final amounts of decomposed C as % of initial litter C-mass versus mg internal N per g-1 litter (Fig.10). In order to make the results comparable to the latter case, a regression was computed between the two variables in order to have a complete decomposition for low values of N.

Only results from temperate and boreal forests litter type were shown. Similarly to the case study with increasing external N, an almost linear trend was obtained between the two variables. Considering the effect of internal N content, when an initial amount of N litter is higher, decomposition occur faster since the metabolic compounds represent an important fraction of litter,

which immobilize less mineral N from the surroundings. Net N mineralization starts earlier, increasing the availability of inorganic N in the system. Since we studied a closed system, the N-inhibitory effect on the litter decomposition leads to a higher mass retention.

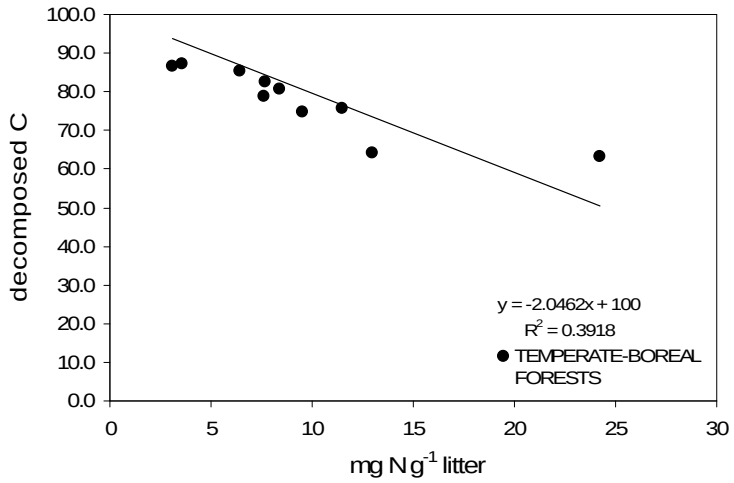

Fig 10 Decomposed C as % reported versus internal litter N concentration expressed as mg N g-1 litter. Data represent only litter data from broadleaves and conifers of the temperate areal.

According to the slope of the regression, their is a potential accumulation of 2% of C sequestered for a unitary increase of internal N. Yet, the obtained value only represents a potential in conditions where saturation does not occur. In natural ecosystems losses (leaching,...) and the interaction with plants regulate mineral N availability. However the model results are in line with the ones reported by Berg and Meetnemayer (2002) who obtained a value of 3.2% C accumulated for a unit increase of N in the litter (mg Ng-1 litter). In their analysis, a linear relationship was obtained between the initial litter-N concentration and the final mass loss obtained by extrapolation of the limit decomposition for several type of litter-bag studies (n=106). Their experimental investigations were conducted in several forest sites covering an European area from Mediterranean to Scandinavian regions, hence implicitly including climate interaction. In fact, compared to our case, they estimated a lower mass loss as litter-N increased.

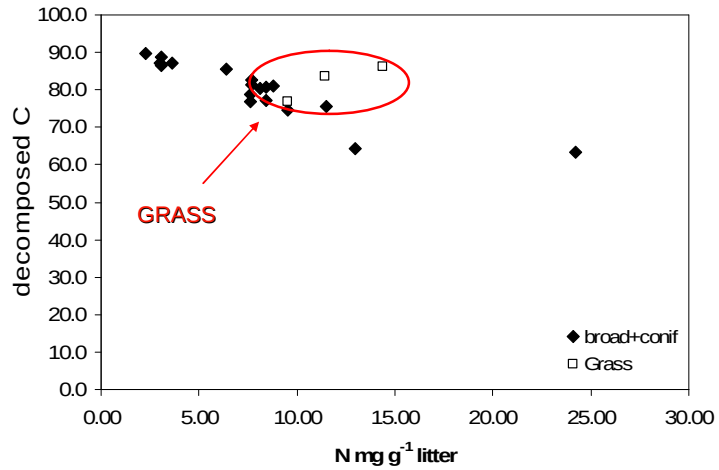

Fig 11 Decomposed C as % reported versus internal litter N concentration. Data are reported clustering litter from broadleaves and conifers for different climatic areas, and grassland.

When adding litter data from Mediterranean and tropical areas, the behaviour follows the pattern observed previously, since it is the litter chemical characteristics that drives the regulation among decomposition stages. The only exception to this rule correspond to grassland litter, the outliers in our study. For this type of ecosystem, the relationship is not hold due the low amount of lignin that is likely to lower the N effect on the final size of the recalcitrant pool (Fig. 11), while the cellulose fraction modulate the positive response to accumulated mineral N. The higher dispersion of data with initial N content is linked to the percentage of lignin content that contribute to the amount of C sequestration.

When the decomposition rates in the model are changed, in order to simulate the climatic effect of temperature and soil moisture, the linear trend is simply steeper when lowering the decomposition constant, the opposite when increasing the rates. For instance, when decomposition runs faster, the N microbial demand against N availability is higher compared to the other cases; hence simply the suppression takes place weakly. The reasons lie in the formulation for temperature and moisture constraints, that is multiplicative as in several models, and it is applied equally to all C pools, while C pools may not respond in the same way to climatic conditions. This can thus not be the correct modelling framework to reproduce the behaviour observed by some authors, who observed a bigger recalcitrant carbon pool in the higher temperature site or for higher temperature incubation (Dalias et al. 2001; Berg and Meentemayer 2002; Coûteaux et al.2001). For example, Dalias (2001) underlined how temperature could have a different type of control depending on the type of C substrate which is degradated i.e. cellulose degradation is promoted by temperature, while recalcitrant-C degradation is not affected. However, in other field experiments the same trend were

not observed when simulating an increase of temperature among a natural gradient (Coûteaux et al.2002, Berg et al.2003). In these studies it was underlined how the site characteristics were also likely to contribute to drive the decomposition of litter. Factors such as autochthonous soil microbial community and texture, when considering under the same climatic conditions, may control the soil response, the mineral N availability and its diffusion. Finally, as a matter of speculation, we may suppose that if higher temperatures lead to a prime of organic matter mineralization and a release of N from the old organic layers, this availability could in turn have a positive feedback on the C stock when the surplus of mineralized N diffuses in the other layers, in the upper as in the lower layers, affecting the overall SOM decomposition.

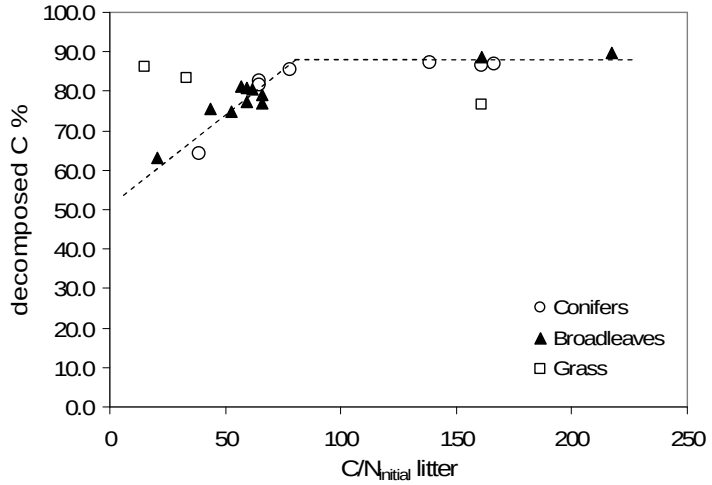

Fig 12 Decomposed C as % reported versus initial C to N ratio of litter. Data are reported clustering litters from broadleaves, conifers and grassland.

We tried to apply the result obtained by the linear relationships analogously as Berg (Berg and Dise 2003) to the case of the N fertilization experiment carried out in four mixed forests in Michigan (U.S.) subjected to 3 g N m^{-2} y^{-2} fertilization rate (Pregitzer et al.2008). The main findings after 10 years of N amendments reported by Pregitzer (2008) was that the simulated N deposition did not affect litter turnover or production, nor belowground or aboveground, maintaining an annual value of about 352 g C m^{-2} throughout the investigation. The experimental evidences revealed an increase of litter N concentration from 0.8 to 1% coupled to an increase of 690 g Cm^{-2} on average in the first 10 cm of the soil; total soil N values were reported including both organic and mineral N. Although the high C accumulation was statistically significant in one of the four experimental sites, an increase of SOC was not apparent. Considering the increase of leaf N, this plant response could justify, after the simple relation proposed, only a 4% retention of the total litterfall in 10 years. It is likely that increasing mineral N affected significantly the decomposition of the old (previously) accumulated organic matter. Because the soil scheme is based on a bucket model, it was not

possible to simulate the humus build-up when trying to apply the model to forest floor and soil maintaining the same parameter optimized on the litterbags studies. For instance the phenomenon of humus build-up refers to the unbalance occurring between litter fall on the soil and SOM decomposition due to the high recalcitrance that does not allow a complete degradation. Hence the accumulation of old organic matter in the deepest layers and the accumulation in these layers of inorganic N could explain the dynamics of the large undecomposed organic matter (Berg et al. 1998).

However, although the relation of the final stage of decomposition on initial litter N concentration, accumulation of mineral N and final stock of organic C was shown in this section (and in the previous one); the absence of a vertical development of the model is likely to make it impossible to predict the accumulation of humus. Especially, as long as an optimization of the parameters is not achieved.

3.7 Conclusions

A model of litter decomposition and organic C accumulation has been developed under the condition of increasing soil N availability emphasizing the role of litter chemistry and microbial activity.

The proposed model, focussing on the N cycle and its feedback on the C-cycle, showed that the total inorganic N may be used explicitly as a proxy of the variable controlling microbial community and its activity within C-cycle, bypassing the question of whether N has a biological rather than a chemical effect. In particular the model takes into account the most important parameters involved in soil biochemical processes avoiding the complexity of a mathematical formulation required at a more mechanicistic level. At the same time it returns the experimental evidences in particular with regards to the way it influences microbial decomposition rates and may be used over a wide range of C/N soil ratio.

Although the model would need a calibration on a larger set of data in order to obtain a better parameter optimization and to characterise the errors associated with the parameter estimate, it is able to reproduce the experimental evidences under increasing N availability due to atmospheric pollution and increasing soil temperature.A change of chemical characteristics of litter due to climate change, including a shift in species composition could not be described in this way. This makes us confident in the working hypothesis behind the model formulation and in the potential use of this soil module coupled to a vegetation model.

The tests of the model have showed two potential limits for C-litter accumulation on long temporal scale for the temperate forests, without taking into account climatic control but only the influence of the litter chemistry and of internal and external N, supported by the main influence of recalcitrance

on response to N addition. The results obtained are in line with data reported in literature by other authors showing the same linear relationship.

The results would suggest that litter with an initial higher N concentration and a higher soil N retention capability in mineral form may have a negative feedback on decomposing litter and on the accumulation of organic matter when recalcitrance becomes important. In particular an important aspect underlined is the potential for inorganic N to offset the C-loss due to a higher SOM mineralization, and hence the following release of N, induced by increased temperature due to climate change.

Neglecting in first approximation the direct climatic influence on decomposition rates, but focussing on C-N interaction, the critical issue appears to be the potential for soil saturation in terms of N accumulation. The point is that there is a biochemical limit for N accumulation. Theoretically it could be expected that the limit C/N of soil is the microbial C/N ratio. But the system may retain the mineral N in abiotic form or reach a condition of saturation before that value due to interaction with plants and losses from the system.

Finally, the model is very sensitive to the vertical development of the soil profile, since it is not able to reproduce the humus build-up that has been observed in several chronosequencing studies in north European forests. Actually we worked with an average amount of N in the soil, while the higher the depth the soil, the higher the amount of the N and the age of organic matter. The model has been calibrated and evaluated for the top soil. However, with a suitable parameters optimization and a larger set of data, keeping the same mathematical formulation should make the model reliable torepresent quite robustly the humus accumulation as a function of soil depth.

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References

Aber, J.D., Melillo, J.M., McClaugherty, C.A., 1990. Predicting long-term patterns of mass loss, nitrogen dynamics, and soil organic matter formation from initial fine litter chemistry in temperate forest ecosystems. Can. J. Bot. 68, 2201–2208.

Aber, J., McDowell, W., Nadelhoffer, K., Magill, A., Berntson, G., Kamakea, M., McNulty, S., Currie, W., Rustad, L., Fernandez, I., 1998. Nitrogen Saturation in Temperate Forest Ecosystems, Hypoheses revisited. Bioscience 48, 921-934.

Aber, J.D., Goodale, C.L., Ollinger, S.V., Smith, M.L., Magill, A.H., Martin, M.E., Hallet, R.A., Stoddard, J.L., 2003. Is nitrogen deposition altering the nitrogen status of northeastern forests? BioScience 53, 375–389.

Barraclough, D., 1997. The direct or MIT route for nitrogen immobilization: a ^{15N} mirror image study with leucine and glycine. Soil Biology & Biochemistry 29, 101-108.

Berg, B., McClaugherty, C., 1987. Nitrogen release in relation to disappearance of lignin. Biogeochemistry 4, 219-224.

Berg, B., Virzo De Santo, A., Rutigliano, F.A., Fierro, A., Ekbohm, G., 2003. Limit values for plant litter decomposing in two contrasting soils—influence of litter elemental composition. Acta Oecologica 24, 295–302.

Berg, B., Matzner, E., 1997. Effect of N deposition on decomposition of plant litter and soil organic matter in forest ecosystems. Environ. Rev. 5, 1-25.

Berg, B., Meentemeyer, V., 2002. Litter quality in a north European transect versus carbon storage potential. Plant and Soil 242, 83–92.

Blackwood, C.B., Waldrop, M.P., Zak, D.R., Sinsabaugh, R.L., 2007. Molecular analysis of fungal communities and laccase genes in decomposing litter reveals differences among forest types but no impact of nitrogen deposition. Environmental Microbiology 9, 1306-1316.

Blagodatsky, S.A., Richter, O., 1998. Microbial growth in soil and nitrogen turnover: a theoretical model considerino the activity state of microorganisms. Soil Biology & Biochemistry 30, 1743-1755.

Brady N.C., Wail R.R., 1999. The nature and properties of soils, 12th edn. Prentice-Hall, New Jersey.

Caldwell, B.A., 2005. Enzyme activities as a component of soil biodiversity: A review. Pedobiologia 49, 637-644.

Chen, J., Stark, J.M., 2000, Plant species effects and carbon and nitrogen cycling in a sagebrush-crested wheatgrass soil Soil Biology & Biochemistry 32, 47-57.

Chertov, O.G., Komarov,A.S., 1997. SOMM: a model of soil organic matter dynamics. Ecological Modelling 94, 177-189.

Chiellini, E., Corti, A., D'Antone, S., Billingham, N.C., 2007. Microbial biomass yield and turnover in soil biodegradation tests: carbon substrate effects. Journal of Polymers and the Environment 15, 169-178.

Cochran, V.L., Horton, K.A., Cole, C.V., 1988. An estimation of microbial death rate and limitations of N or C during wheat straw decomposition. Soil Biology & Biochemistry 20, 293–298.

Cortez, J., Demard, J.M., Bottner, P., Monrozier, L.J., 1996. Decomposition of Mediterranean leaf litters: A microcosm experiment investigating relationships between decomposition rates and litter quality. Soil Biology & Biochemistry 28, 443–452.

Coûteaux, M., Bottner, P., Berg, B., 1995. Litter decomposition, climate and litter quality. Tree 10, 63-66.

Couteaûx, M.M., Bottner, P., Anderson, J.M., Berg, B., Bolger, T., Casals, P., Romanya, J., Thiery, J.M., Vallejo, V.R., 2001. Decomposition of ^{13}C-labelled standard plant material in a latitudinal transect of European coniferous

forests: Differential impact of climate on the decomposition of soil organic matter compartments. Biogeochemistry 54, 147–170.

Couteaûx, M.M., Sarmiento, L., Bottner, P., Acevedo, D., Thiery, J.M., 2002. Decomposition of standard plant material along an altitudinal transect (65-3968 m) in the tropical Andes. Soil Biology & Biochemistry 34, 69-78.

Dalias, P., Anderson, J.M., Bottner, P., Coûteaux, M.M., 2001. Long-term effects of temperature on carbon mineralisation processes. Soil Biology & Biochemistry 33, 1049-1057.

Davidson, E.A., Chorover, J., Dail, D.B., 2003. A mechanism of abiotic immobilization of nitrate in forest ecosystems: the ferrous wheel hypothesis. Global Change Biology 9, 228-236.

DeForest, J.L., Zak, D.R., Pregitzer, K.S., Burton,A.J., 2004. Atmospheric Nitrate Deposition, Microbial Community Composition, and Enzyme Activity in Northern Hardwood Forests. Soil Sci. Soc. Am. J. 68, 132–138.

Demoling, F., Nilsson, L.O., Bååth, E., 2008. Bacterial and fungal response to nitrogen fertilization in three coniferous forest soils. Soil Biology & Biochemistry 40, 370–379.

De Santo, A.V., Rutigliano, F.A., Berg, B., Fioretto, A., Puppi, G., Alfani, A., 2002. Fungal mycelium and decomposition of needle litter in three contrasting coniferous forests. Acta Oecol. 23, 247–259.

de Vries, W.,Solberg, S., Dobbertin, M., Sterba, H., Laubhann, D., van Oijen, M., Evans, C., Gundersen, P., Kros, J., Wamelink, G.W.W., Reinds, G.J., Sutton, M.A., 2009. The impact of nitrogen deposition on carbon sequestration by European forests and heathlands. Forest Ecology and Management 258, 1814–1823.

Dommergues, Y., Mangenot, F., 1970. Ecologie Microbienne Du Sol. Masson, Paris.

Fang, C., Smith, P., Smith, J.U., Moncrieff, J.B., 2005. Incorporating microorganisms as decomposers into models to simulate soil organic matter decomposition. Geoderma 129, 139–146.

Flaig, W., 1971. Organic compounds in soil. Soil Science 111, 19-33.

Fog, K., 1988. The effect of added nitrogen on the rate of decomposition of organic matter. Biological Reviews 63, 433–462.

Franklin, O., Högberg, P., Ekblad, A., Göran, Ågren, G.I., 2003. Pine Forest Floor Carbon Accumulation in Response to N and PK Additions: Bomb ^{14}C Modelling and Respiration Studies. Ecosystems 2003 6, 644–658.

Frey, S.D., Knorr, M., Parrent, J.L., Simpson, R.T., 2004. Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. Forest Ecology and Management 196 (2004) 159–171.

Gallardo, A., Merino, J., 1993. Leaf decomposition in two Mediterranean ecosystems of southwest Spain: Influence of substrate quality. Ecology 74, 152–161.

Gallo, M., Amonette, R., Lauber, C., Sinsabaugh, R.L., Zak, D.R., 2004. Microbial Community Structure and Oxidative Enzyme Activity in Nitrogen-Amended North Temperate Forest Soils. Microbial Ecology, 48, 218–229.

Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., Karl, D.M., Michaels, A.F., Porter, J.H. Porter, Townsend, A.R., Vörösmarty, C.J., 2004. Nitrogen cycles: past, present, and future. Biogeochemistry 70,153–226.

Garhooee, F.R., 1998. Impacts of elevated atmospheric CO_2 on litter quality, litter decomposability and nitrogen turnover rate of two oak species in a Mediterranean forest eco system. Global Change Biology 4, 667-677.

Grandy, S.A., Sinsabaugh, R.L., Neff, J.C., Stursova, M., Zak, D.R., 2008. Nitrogen deposition effects on soil organic matter chemistry are linked to variation in enzymes, ecosystems and size fractions. Biogeochemistry 91, 37–49.

Gundersen, P., Emmett, B.A., Kjønaas, O.J., Koopmans, C.J., Tietema, A., 1998. Impact of nitrogen deposition on nitrogen cycling in forests: a synthesis of NITREX data. Forest Ecology and Management 101, 37-55.

Hadas, A., Sofer, M., Molina, J.A.E., Barak, P., Clapp, C.E., 1992. Assimilation of nitrogen by soil microbial population: NH_4 versus organic N. Soil Biology & Biochemistry 24, 137–143.

Hadas, A., Parkin, T.B., Stahl, P.D., 1998. Reduce CO_2 release from decomposing wheat straw under N-limiting conditions: simulation of carbon turnover. European Journal of Soil Science 49, 487-494.

Hagedorn, F., Spinnler, D., Siegwolf, R., 2003. Increased N deposition retards mineralization of old soil organic matter. Soil Biology & Biochemistry 35, 1683–1692.

Harmon, M.E., Silver, W.S., Fasth, B., Chen, H., Burkez, I.C., Parton, W.J., Hart, S.C., Curriek, W.S., LIDET, 2009. Long-term patterns of mass loss during the decomposition of leaf and fine root litter: an intersite comparison. Global Change Biology 15, 1320–1338.

Hart, S.C., Firestone, M.K., Paul, E.P., 1992. Decomposition and nutrient dynamics of ponderosa pine needles in a Mediterranean-type climate. Can. J. For. Res. 22, 306–314.

Henriksen, T.M., Breland, T.A., 1999. Nitrogen availability e€ects on carbon mineralization, fungal and bacterial growth, and enzyme activities during decomposition of wheat straw in soil Soil Biology and Biochemistry 31, 1121-1134.

Hyvönen, R., Persson, T., Andersson, S., Olsson, B., Ågren, G.I., Linder, S., 2008. Impact of long-term nitrogen addition on carbon stocks in tree and soils in northern Europe. Biogeochemistry 89, 121-137.

Hobbie, S.E., 2000. Interactions between litter lignin and soil nitrogen availability during leaf litter decomposition in a Hawaiian montane forest. Ecosystems 3, 484–494.

Högberg, M.N., Högberg, P., Myrold, D.M., 2007. Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three? Oecologia 150, 590–601.

Hungate, B.A., Dukes, J.S., Shaw, M.R., Luo, Y., Field, C.B., 2003. Nitrogen and Climate Change. Nature 302, 1512.

Kirschbaum M.U.F., Paul K.I., 2002. Modelling C and N dynamics in forest soils with a modified version of the Century model. Soil Biology & Biochemistry 34, 341-354.

Knorr, M., Frey, S.D., Curtis, P.S., 2005. Nitrogen additions and litter decomposition: a meta-analysis. Ecology 86, 3252-3257.

Janssens, I.A., Sebastiaan, L., 2009. Carbon cycle: Nitrogen's carbon bonus. Nature Geoscience 2, 309-370.

Lekkerkerk, L., Lundkvist, H., Ågreen, G., 1990. Decomposition of heterogeneous substrates; and experimental investigation of a hypothesis on substrate and microbial properties. Soil Biology & Biochemistry 22, 161-167.

Li, X., Meixner, T., Sickman, J.O., Miller, A., SchimeL, J.P., Melack, J.M., 2006. Decadal-scale dynamics of water, carbon and nitrogen in a California chaparral ecosystem: DAYCENT modeling results Biogeochemistry 77, 217–245.

Liu, J., Price, D.T., Chen, J.M., 2005. Nitrogen controls on ecosystem carbon sequestration: a model implementation and application to Saskatchewan, Canada. Ecological Modelling 186, 178–195.

Lynch, J. M. 1987. Lignocellulolysis in Composts. In: Compost: Production, Quality and Use (M. De Bertoldi, M. P. Ferranti, P. L.'Hermite, and F. Zucconi, ed.). Elsevier Applied Science, New York. 853 pp.

Macdonald, J.A., Dise, N.B., Matzner, E., Armbruster, M., Gundersen, P., 2002. Nitrogen input together with ecosystem nitrogen enrichment predict nitrate leaching from European forests. Global Change Biology 8, 1028-1033.

Magill A.H., Aber, J.D., Hendricks, J.J., Bowden, R.D., Melillo, J.M., Steudler, P.A., 1997. Biogeochemical response of forest ecosystems to simulated chronic nitrogen deposition. Ecological application, 7(2), 402-415.

Magill, A.H., Aber, J.D., 1998. Long-term effects of experimental nitrogen additions on foliar litter decay and humus formation in forest ecosystems. Plant and Soil 203, 301–311.

Magill, A.H., Aber, J., 2000. Dissolved organic carbon and nitrogen relationships in forest litter as affected by nitrogen deposition. Soil Biology & Biochemistry 32, 603-613.

Magill, A.H., Aber, J.D., Currie, W.S., Nadelhoffer, K.J., Martin, M.E., McDowell, W.H., Melillo, J.M., Steudler, P., 2006. Ecosystem response to 15 years of chronic nitrogen additions at the Harvard Forest LTER, Massachusetts, USA Forest Ecology and Management 196, 7–28.

Magnani, F., Mencuccini, M., Borghetti, M., Berbigier, P., Berninger, F., Delzon, S., Grelle, A., Hari, P., Jarvis, P.G., Kolari, P., Kowalski, A.S., Lankreijer, H.L., Law, B.E., Lindroth, A., Loustau, D., Manca, G., John, B., Moncrieff, J.B., Rayment, M., Tedeschi, V., Valentini, R., Grace, J., 2007. The human footprint in the carbon cycle of temperate and boreal forests. Nature 447, 848-852.

Mäkipää, R., 1995. Effect of nitrogen input on carbon accumulation of boreal forest soils and ground vegetation. Forest Ecology and Management 79, 217-226.

Manzoni, S., Jackson, R.B., Trofymow, J.A., Porporato, A., 2008. The Global Stoichiometry of Litter Nitrogen Mineralization. Science 321, 684-686.

Manzoni, S., Porporato, A., 2009. Soil carbon and nitrogen mineralization: Theory and models across scales. Soil Biology & Biochemistry xxx, 1–25.

Melillo, J.M., Aber, J.D., Muratore, J.F., 1982. Nitrogen and Lignin Control of Hardwood Leaf Litter Decomposition Dynamics. Ecology 63, 621-626.

Melillo, J.M., Steudler, P.A., Aber, J.D., Newkirk, K., Lux, H., Bowles, F.P., Catricala, C., Magill, A., Ahrens, T., Morrisseau, S., 2002. Soil warming and carbon-cycle feedbacks to the climate systems. Science 298, 2173–2176.

Micks, P., Aber, J.D., Boone, R.D., Davidson, E.A., 2004. Short-term soil respiration and nitrogen immobilization response to nitrogen applications in control and nitrogen-enriched temperate forests. Forest Ecology and Management 196, 57–70.

Minderman, G., 1968. Addition, Decomposition and accumulation of organic matter in forests. J. of Ecology 56, 355-362.

Mo, J., Zhang, W., Zhu, W., Gundersen, P., Fang, Y., Li, D., Wang, H., 2008. Nitrogen addition reduces soil respiration in a mature tropical forest in southern China. Global Change Biology 14, 403-402.

Molina, J.A.E., Clapp, C.E., Shaffer, M.J., Chichester, F.W., Larson, W.E., 1983. NCSOIL, a model of nitrogen and carbon transformation in soil: description, calibration and behaviour. Soil Sci. Soc. Am. J. 47, 85-91.

Moorhead, D.L., Sinsabaugh, R.L., 2006. A theoretical model of litter decay and microbial interaction. Ecological Monographs 76, 151–174.

Mueller, T., Jensen, L.S., Hansen, S. & Nielsen, N.E. (1996) Simulating soil carbon and nitrogen dynamics with the soil–plant–atmosphere system model DAISY. Evaluation of Soil Organic Matter

Models (ed. by D.S. Powlson, P. Smith and J.U. Smith), pp. 275–282. Springer Verlag, Berlin.

Nabuurs, G.J., O. Masera, K. Andrasko, P. Benitez-Ponce, R. Boer, M. Dutschke, E. Elsiddig, J. Ford-Robertson, P. Frumhoff, T. Karjalainen, O. Krankina, W.A. Kurz, M. Matsumoto, W. Oyhantcabal, N.H. Ravindranath, M.J. Sanz Sanchez, X. Zhang, 2007: Forestry. In Climate Change 2007: Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [B. Metz, O.R. Davidson, P.R. Bosch, R. Dave, L.A. Meyer (eds)], Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Nadelhoffer, K., et al., 2004. Decadal-scale fates of N-15 tracers added to oak and pine stands under ambient and elevated N inputs at the Harvard Forest (USA). Forest Ecology and Management 196, 89–107.

Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G., Renella, G., 2003. Microbial diversity and soil functions. European Journal of Soil Science 54, 655–670.

Neff, J.C., Townsend, A.R., Gleixner, G., Lehman, S., Turnbull, J., Bowman, W.D., 2002. Variable effects of nitrogen additions on the stability and turnover of soil carbon. Nature 419, 915–917.

Nicolardot, B. Molina, J.A., Allard, M.R., 1994. C and N fluxes between pools of soil organic matter: model calibration with long-term incubation data. Biology & Biochemistry 26, 235-243.

Parton, W.J., Schimel, D.S., Cole, C.V. & Ojima, D.S. 1987. Analysis of factors controlling soil organic matter levels in Great Plains grasslands. Soil Science Society of America Journal 51, 1173-1179.

Parton, W.J., Stewart, J.W.B., Schimel, D.S., 1988. Dynamics of C,N,P and S in grassland soils: a model. Biogeochemistry 5, 109-131.

Parton, W.J., Scurlock, J.M.O., Ojima, D.S., Gilmanov, T.G., Scoles, T.G., Schimel, D.S., Kirchner, T., Menaut, J.C., Seastedt, T., Garcia Moya, E., Kamnalrut Apinan, Kinyamario, J.I., 1993. Observations and modeling of biomass and soil organic matter dynamics for the grassland biome worldwide. Global Biogeochemical Cycles 7, 785–809.

Parton, W.J., Rasmussen, P.E., 1994. Long-term effects of crop management in wheat– fallow: II. Century model simulations. Soil Sci. Soc. Am. J. 58, 530– 536.

Pepper, D.A., Del Grosso, R.E., McMurtrie, R.E., Parton, W.J., 2005. Simulated carbon sink response of shortgrass steppe, tallgrass prairie and forest ecosystems to rising [CO_2], temperature and nitrogen input. Global Biogeochemical Cycles, 19, ??

Pregitzer, K.S., Burton, A.J., Zak, D.R., Talhelm, A.F., 2008. Simulated chronic nitrogen deposition increases carbon storage in Northern Temperate forests. Global Change Biology 14, 142–153.

Puri, G., Ashman, M.R., 1999. Microbial immobilization of ^{15}N-labelled ammonium and nitrate in a temperate woodland soil. Soil Biology & Biochemistry 31, 929-931.

Reich, P.B., Hungate, B.A., Luo, Y., 2006. Carbon-Nitrogen Interactions in Terrestrial Ecosystems in Response to Rising Atmospheric Carbon Dioxide. Annu. Rev. Ecol. Evol. Syst. 37, 611-636.

Reay, D.S., Dentener, F., Smith, P., Grace, J., Feeley, R.A., 2008. Global nitrogen deposition and carbon sink. Nature Geoscience 1, 430-437.

Roberts, P., Stockdale, R., Khalid, M., Iqbal, Z., Jones D.L., 2009, Carbon-to-nitrogen ratio is a poor predictor of low molecular weight organic nitrogen mineralization in soil. Soil Biology & Biochemistry 41, 1750-1752.

Rustad, L.E., Campbell, J.L., Marion, G.M., Norby, R.J., Mitchell, M.J., Hartley, A.E., Cornelissen, J.H.C., Gurevitch, J., 2001. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental eco system warming. Oecologia 126, 543–562.

Shibu, M.E., Leffelaar, P.A., Van Keulen, H., Aggarwal, P.K., 2006. Quantitative description of soil organic matter dynamics—A review of approaches with reference to rice-based cropping systems. Geoderma 137, 1–18.

Schimel D.S., Braswell Jr E.A., Holland R., Mc-Keown D.S et al., 1994. Climatic, edaphic and biotic controls over storage and turnover of carbon in soils. Global Biochemical Cycle 8, 279-293.

Schimel, J.P., Bennett, J., 2004. Nitrogen mineralization: challenges of a changing paradigm Ecology, 85, 591–602.

Schulten, H.R., Schnitzer, M., 1998. The chemistry of soil organic nitrogen: a review. Biology and fertility of soils 26, 1-15.

Sinsabaugh, R.L., Carreiro, M.M., Repert, D.A., 2002. Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. Biogeochemistry 60, 1–24.

Sinsabaugh, R.L., Gallo, M.E., Lauber, C., Waldrop, M.P., Zak, D.R., 2005. Extracellular enzyme activities and soil organic matter dynamics for northern hardwood forests receiving simulated nitrogen deposition. Biogeochemistry, 75: 201–215.

Six, J., Conant, R.T., Paul, E.A., Paustian, K., 2002. Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. Plant and Soil 241, 155–176,

Smith, P., Powlson, D.S., Smith, J.U., Elliot, E.T., 1997. Evaluation and comparison of soil organic matter models using long term datasets. Geoderma 81, 1 –255.

Smith, J.U., Smith, P., Addiscott, T. 1996. Quantitative methods to evaluate and compare soil organic matter (SOM) models. In: Evaluation of Soil Organic Matter Models using Existing Longterm Datasets (eds D.S. Powlson, P. Smith & J.U. Smith), pp. 181±199. Springer-Verlag, Berlin.

Smith, E.P., Kenneth, A.R., 1995. Model goodness-of-fit analysis using regression and related techniques. Ecological Modelling 77, 49-64.

Södeström, B., Bååth, E., Lundgren, B., 1983. Decrease in soil microbial activity and biomasses owing to nitrogen amendments. Can. J. Microbiol. 29, 1500-1506.

Sutton, M.A., Simpson, D., Levy, P.E., Smith, R.I., Reis, S., Van Oijen, M., De Vries, W., 2008. Uncertainties in the relationship between atmospheric nitrogen deposition and forest carbon sequestration Global Change Biology 14, 2057–2063.

Taylor, B.R., Prescott, C.E., Parsons, W.J.F., Parkinson, D., 1991. Substrate control of litter decomposition in four Rocky Mountain coniferous forests. Can. J. Bot. 69, 2242–2250.

Tirukkumaran, C.M., Perkinson, D., 2000. Microbial respiration, biomass, metabolic quotient and litter decomposition in a lodgepole pine forest floor amended with nitrogen and phosphorous fertilizers. Soil Biology & Biochemistry 32, 59-66.

Van Veen, J.A., Ladd, J.N., Frissel, M.J., 1984. Modelling C and N turnover through the microbial biomass in soil. Plant Soil 76, 257–274.

Van Veen, J.A., Ladd, J.N., Amato, M., 1985, Turnover of carbon and nitrogen through the microbial biomass in a sandy loam and a clay soil incubated with ^{14}C-glucose and ^{15}N-$(NH_4)_2SO_4$ under different moisture regimes. Soil Biology & Biochemistry 17, 747-756.

Zourkoura, A., Mamolos, A.P., Kalburtji, K.L., 2003. Decomposition of dominant plant species litter in a semi-arid grassland. Applied Soil Ecology 23, 13–23.

4 SOM DECOMPOSITION MODEL and MICROBIAL SUBSTRATE PREFERENCES: A QUALITATIVE STUDY

Keywords: *soil mixed substrates, microbial community, ecological goal.*

4.1 Introduction

Soil is a complex system characterized by a highly spatial heterogeneity concerning the microbial populations and the organic matter (OM) mixture with mineral matrix. Thanks to soil texture and the occurrence of specific conditions at macro and micropores scale, microorganisms show a spatial organization exhibiting wide specie richness (Ettema et al.,2002) and constitute a synergistic interacting community. Markedly, soil-microorganism complex can be considered as a self-organizing system that works in order to gain an optimal environment for living and operates at physical and chemical level (Young and Crawford 2004). Abiotic (soil water content, temperature, soil texture, structure etc) and biotic factors (i.e. microbial community structure, nutrient status, soil biological diversity etc) affect microbial performances in a non linear way and microbial world represents thus a fascinating topic for soil scientists. However the understanding of soil functions mediated by the single microbial specie rather than microbial community structure is still an open question due to the difficulties to link experimentally the two items (Nannipieri et al., 2003). The gaps in our knowledge are critical since the role of soil microbes is of utter importance for the natural ecosystems and involve the plant productivity, the soil health, resilience and recovery capability toward external disturbances. Moreover microbes have a higher turnover and are highly sensitive on the short temporal scales compared to other biotic compartment such as the vegetation. Microorganisms constitute hence sensitive indexes toward changes of either abiotic or biotic drivers induced by climate changes or anthropogenic activities.

The microbial colonization operates at different levels, as hot spots in the micropores or at higher scales with regards to litter constituents; soil is hence characterized by the coexistence of different microbial species growing on mixtures of substrates and exhibit different degree of activity.

In literature the microbial growth mechanism in the presence of more than one substrate has been investigated mainly in laboratory batch and chemostat cultures, by medium of simple carbon (C) compounds supply, i.e. glucose, aminoacids, and pure microbial specie. At this scale of observation it was possible to detect a diaustic or sequential growth, thus microbes are able to growth on one substrate only and then to shift to the second one once the first is exhausted. This occurs after a time lag where microbes reorganize themselves. In other cases it has been shown a simultaneous growth of microbes on the different compounds. At this scale a major control can be operated in order to drive the experimental condition for the assimilation and growth of the single cell rather than the unit of biomass. Contrarily in the soil many C-sources are available among heterotrophic microbial species and different nutrients and physical-chemical conditions. On one hand microorganisms have the opportunity for different substrates choice, on the other hand when upscaling and enlarging the spatial scale of observation the complexity and heterogeneity of the system can lead to a

smoothness of the different signals. Indeed the ecological function of interest performed by soil microorganisms is the decomposition of litter soil organic matter (SOM). However the high soil redundancy and the several output in terms of carbon dioxide (CO_2) respired, may confound some ecological information that could have a significant impact on long temporal scales such as C losses induced by the priming effect or carbon-nitrogen interaction (Blagodastakya and Kuzyakov 2008).

Under a biochemical point of view, the decomposition activity of the several C compounds is mediated by enzymatic catalytic activity (Nannipieri et al.2003). The production and the efficiency of C-enzymes is the first limiting step for organic matter decomposition and microbial utilization of the resources. On one hand the soil enzymatic study suffers of the problems related to the nature of enzymes, intracellular or extracellular that can be absorbed on mineral matrix, inducible and constitutive enzymes that can be produced independently by the presence of the substrates. On the other hand some enzymatic families share common substrates or several microbial families accomplish the same ecological functions thus allow us to overcome the difficulties linked to the knowledge of microbial structure and single functions.

Sinsabaugh and Moorhead (2000) showed how it was possible to relate the degradation of some litter compounds and the activity of specific extracellular enzymes, i.e. lignocellulosic activity exhibited a significant correlation with instantaneous mass loss rates. Besides, Sinsabaugh (1994) and Allison (2005) showed how the enzymatic production follows micro-economic rules. For example the first author showed experimentally and by medium of a model that release of enzymes to the soil is inversely proportional to the availability of nutrients, i.e. nitrogen (N) and phosphorous in a litter decomposition model, and Allison and Vitousek detected how the production of enzymes for degradation of more recalcitrant substrates may be reduced when labile resources are available. Indeed microbes prefer the easily available C substrates, the quantity of which is determined in the field, by the litter input and root rhizodeposition processes (Trumbore et al.,1990; Cheng 1999; Bahn et al.,2006). The microbial substrate preference for easily decomposable compounds with a consequent retardation of native organic matter decomposition has been proposed by other authors in literature (Craine et al.,2006; Blagodastakya and Kuzyakov 2008).

At the current state, adaptive models based on choice of different resources are well known. On one hand population behaviour models are mainly applied to superior organisms (i.e. mammals feeding on several food patches). With regards to plant system (Givnish 1986), optimization mechanisms concerning a particular ecological goal under specific biological constrains have been applied with good results to investigate the plant response to global change considering effects such as CO2 fertilization and N availability (Franklyn 2007; Dewar et al.2009). Concerning microbial population, several complex and mechanicistic models exist that investigate the behaviour of microorganisms growing on mixed substrates that is a topic of interest in the field of

bioengineering, i.e. bioremediation of mixed pollutants etc. rather than microbiology (Varner and Ramkrishna, 1999; Brandt et al. 2003).

At the scales that involve the soil C-turnover, most of the existing soil process-oriented models do not take in to account microbial community explicitly, but soil biotic component is mainly treated as a dead soil C pool and the decomposition of different substrates is independent by other substrates (no priming effect or suppressive effect) and follows a first order kinetic. However, although such models are easily applicable at large spatial and temporal scale, they are not able to mimic some important biological mechanisms that have a higher frequency, or a small fluctuation in terms of C and N fluxes, but that could have important repercussions on long temporal scale.

The aim of the present work, in its first attempt, is to offer a new perspective to the study of soil C-cycling applying two major modifications to existing models: the substrate preference and the co-limitation of degradation by both substrates and microorganisms. The models presented here take account for the microbial community as a whole and microbial behaviour in term of preferences on mixed substrates (i.e. litter versus native soil organic matter) overcoming the difficulties due to the inscrutability of the several characteristics and functionality of microbial populations. Hence, compared to more detailed models, I explored the consequences of regulating the microbial preferences depending on substrates amount and quality, nutrient status and thus on external variables that do not involve directly biological mechanism at cellular or species level. The proposed formulations for the soil decomposition models, allow to explain with really few parameters some potential phenomena that have been detected in both lab and field experiments. Markedly the best results in the present work were achieved with the model including the maximization of growth mechanisms and the non-linear dependence of decomposition on microbial biomass. The qualitative results of the modified model would support the potential existence of both a positive and a negative priming on the long temporal scale, depending on the substrate choice by microorganisms.

4.2 Materials and methods

4.2.1 Models description

The spatial scale of observation regards from laboratory to plot scale and the temporal scale is the daily. The main structure of the C model is reported in Fig. 1. Microbial community B has been considered as a single individual without considering separately the different species or any taxonomical characterization, although the evidence of a higher biodiversity and the individuation of different interacting functional groups. To avoid an overparameterization of the model, any intermediary step such as extracellular enzymatic catalytic activity exists, between microorganisms and carbon pools C, but substrates are degraded and suddenly assimilated by microbial cell. The microbial gain of C is considered at the net expenses due to degradation, maintenance and biosynthesis, i.e. microbial tissues and enzymes, and every metabolic activity but without considering explicitly every mechanism. The yield coefficients is defined as the ratio of biosynthesis carbon to mineralized carbon.

The model in its simple form does not include hence nor an explicit enzymatic pool (Schimel and Weintraub 2003), that is considered as a constant fraction of microbial pool, or guilds characterized by specific functionality (Fountaine and Barot 2005).

The basic concept behind the present scheme is that soil is a bucket with well mixed organic matter compounds and microbes, despite of the importance of the soil spatial variability and the effects on microbial performances. The C-substances are split in several conceptual pools characterized by different quality, markedly by different capabilities to be degraded by microorganisms.

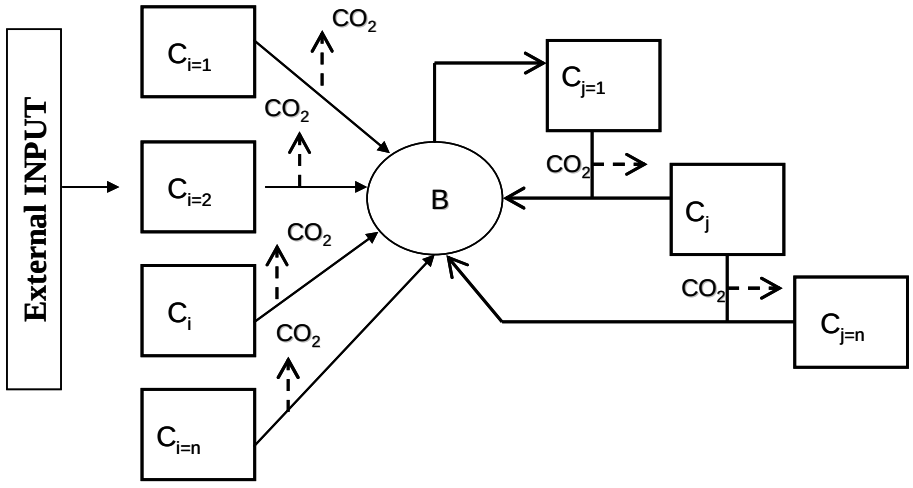

Fig. 1. Scheme of the soil C-model, solid lines represent organic C fluxes between the different pools, dashed lines represent mineralized C due to microbial respiration associated to degradation cost, growth, maintenance and turnover; B is the total microbial biomass.

The substrate quality can depend on both chemical structures of the organic compound that influence microbial enzymatic attack and energetic cost and by limited accessibility due to protection mechanisms adducible to the mineral matrix interaction. Analogously these differentiations may have a correspondence in the light and the heavy C fraction of soil.

A class of substrates C_i are supplied externally (i.e. litter, rizodepositions) and are referred to labile compounds or highly bioavailable substrates. C_j pools are formed internally *via* the microbial activity that leads to the formation of recalcitrant substances than can in turn be included in mineral matrix with the formation of the equivalent of the mineral soil. The first class regards simple or metabolic compounds as water soluble and low molecular weight substances, including sugars, proteins, aminoacids and root exudates, cellulosic compounds or simple phenolic compounds. The classification with regards to particular organic component has been maintained in order to have a direct link with a specific class of enzymes.

As a first attempt, to avoid overparameterization, it has been not been considered the C-mineralization induced by the microbial turnover, but any C released because of catabolic activity is included in the yield coefficient. It has not been assumed any minimum energetic level for microbial activation and enzymatic production or any fixed amount of constitutive enzymes absorbed on mineral the mineral matrix (Allison and Vitousek 2005).

C-models

The mathematical formulation shares a lot of features with other soil models existing in literature.

The mixed model has been implemented in order to consider the feeding of microbial community on the two different type of resources Ci and Cj. The equations are explicitly function of both substrate and microbial biomass in order to include simultaneously the occurrence of a potential limitation to C-degradation due to both microorganisms or resources limitation as well as priming effect (Wurztler and Reichstein 2008). Markedly one model for decomposition of C_i is linear function of microbial biomass following the model used by Porporato and Manzoni (2007) to mimic the more accessibility of substrate and the major control of microorganisms; the second model is an inverse Monod-type equation where the microbial saturation occurs and the maximum decomposition rates is determined by amount of substrates. The degradation of pools C_j follows a classical Monod-type equation with a substrate saturation mechanism and the maximum decomposition activity dependent on the microbial biomass size. Enzymatic compartment is implicitly included in the microbial pool B. K_{ai} and K_i are the decay constants for labile or easily accessible substrate, K_j is the decomposition rate for recalcitrant C or less accessible compounds; K_{si} is the half saturation constant of carbon-substrate saturation, analogously K_{mi}; α the microbial assimilation efficiency (C used for biosynthesis per unit of degraded C); I_i is the external supply to

pools Ci; λ is the microbial turnover. The biological meaning of degradation constants depends on what microbes perceive as a resource available in term of accessibility and efficiency of enzymatic attack. The parameter β indicates the amount of active biomass feeding and growing on a specific C-substrate. It follows the mathematical formulation:

$$\frac{dC_i}{dt} = -K_{ai} B C_i \beta_i + I_i$$

$$\frac{dC_j}{dt} = -K_j B \frac{C_j}{K_{sj} + C_j} \beta_j + \lambda B$$

$$\frac{dB}{dt} = \sum_{i=1}^{n} K_{ai} B \beta_i C_i \alpha_i + \sum_{j=1}^{m} K_j B \beta_j \frac{C_j}{K_{sj} + C_j} \alpha_j - \lambda B$$

This model where the decomposition of both litter and soil organic matter depends linearly on microbial biomass will be referred thereafter as model 1.

In the case of non-linear dependence toward microbial biomass of litter mineralization, the following formulation states:

$$\frac{dC_i}{dt} = -K_i C_i \frac{\beta_i B}{\beta_i B + K_{mi}} + I_i$$

the formulation for microbial growth pool B is thus modified after the new decomposition model for the C_i pools. This second model will be called model 2.

Nitrogen cycle has been included in some simulations and it is computed as the C-fluxes multiplied by the C/N ratio of the source of the C. The entire N is firstly mineralized and then taken up by microbes after the scheme presented in the Chapter 2 where the assimilation of mineral N is determined by the parameter K_{bu}. In the present work organic N assimilation has not been considered.

Determination of β

The working hypothesis assumed that in the temporal window of observation *dt* the equilibrium between microbial community and C-substrates and nutrients in the surroundings is achieved. The dynamic evolution of organic matter degradation is hence the sum of the several equilibrium states. The formulation states that once C-substrates are bio-available, they are immediately degraded and assimilated allowing microbes to growth without any step in the between. The further assumption is that the different substrates are well mixed, although in the soil are patchy distributed, the microbial community has on average the same ability to explore the C-compounds in the surroundings, both size and quality but without needing to move for exploitation.

As a first attempt in order to define parameter β, it has been started from a special case of the input matching principle used in ecology, following the formulation of the cybernetic modelling approach proposed by Varner and Ramkrishna (1999) where, based on microeconomics rules, the maximization of return from resources is determined by the fractional allocation of investment. The input matching law has been widely used to describe the animal distribution on different food choices under specific working conditions (Tregenza 1994; Lessels 1995) and states how consumer distribution depends proportionally on the return from a specific resource. In the cybernetic model (when considering the linear convergent pathway of C-fluxes from several sources Ci) the fractional allocation of critical resource Ri toward a specific path i is equivalent to the fractional return from that pathway as follows:

$$\sum_{i=1}^{n} R_i = const$$

$$\frac{dR_i}{\sum_{i=1}^{n} dR_i} = \frac{dG_i}{\sum_{i=1}^{n} dG_i}$$

where G_i is a reaction rate, i.e. enzymatic activity. The mathematical result derives from the maximization of the sum of the products that are released by the enzymatic activity in a specific time step. However the formulation on the right side of the equation is used to express a control variable that drives the production of a specific enzyme devoted to the degradation of compound Ci. In the present case it has been considered a similar formulation where G_i is the population growth rate per unit of microbial biomass and the fractional allocation of resources can be computed easily and explicitly when the dependence of decomposition is linear in the biomass. Hence for model 1 the following formulation has been used:

$$\beta_i = \frac{dG_i}{\sum_{i=1}^{n} dG_i}$$

With

$$\sum_{i=1}^{n} \beta_i B = B$$

Where β_i is the allocation of resources that is the amount of microbial biomass that allocate C for the production of specific enzymatic family *i* (i.e. cellulase toward cellulolytic compounds; oxidase towards recalcitrant C etc). Actually the enzymatic pool takes a constant fraction of microbial pool hence β_i in the fractional allocation of microbial community B feeding on the different resources C_i.

The formulation states hence that the enzymatic production is considered to be regulated by microbial community in order to allocate resources to produce a specific enzymatic family devoted to degradation of a C*i* or a C*j* substrate. In turn, from a specific pathways *i* or *j*, microbial community has a specific return in terms of C that is invested again for production of biomass and enzymes. G represents thus the return from the single pathway that in the time step *dt* constitutes the net growth rate; *n* constitutes the total number of substrates available. However the present formulation does not allow the maximization of microbial end product, but simply follows the input matching rule.

Under an ecological point of view, βi can be referred to the number of microorganisms feeding on a single substrate rather than the formation of different microbial families or guild growing on a C-compound, the functional expression of which is performed by the specific enzymatic production. The single return from the specific C-compound computed per unit of microbial biomass is thus:

$$dGi = K_{ai} C_i \alpha_i$$

or

$$dG_j = K_j \alpha_j \frac{C_j}{K_{sj} + C_j}$$

depending on the C-source.

Comparing to the original cybernetic model, I neglect hence that a key enzyme must be synthesized before degradation and growth can occur. In the present work the control operates at the level of the decomposition reaction rate that is mediated implicitly by extracellular enzymes (Moorhead and Sinsabaugh, 2000) whilst the detailed expression of mechanisms such as complexation enzyme-substrate or inhibition processes concerning enzymatic production is not considered.

For model 2, a second formulation for expression of βi was used and it is based on the maximization of the microbial growth rate at each single time step. Thus the goal of the microbial community as a single individual is devoted to the same ecological objective: the maximization of the net growth as the sum of the returns of C from the different C sources. In this case a non-linear formulation of microbial biomass has been used, hence the mathematical formulation states as follows:

$$MAX \sum_{k=1}^{n+m} \frac{dB}{dt} = \sum_{i=1}^{n} K_i \frac{\beta_i B}{\beta_i B + K_m} C_i \alpha_i + \sum_{j=1}^{m} K_j B \beta_j \frac{C_j}{K_{sj} + C_j} \alpha_j$$

without constraints on maximum microbial growth rates, but on the amount of microorganisms:

$$\sum_{i=1}^{n+m} \beta_i = 1$$

In this case β_i constitutes the optimal allocation of resources, i.e. enzymatic production and hence microbes on the different compounds. It is expected that microbes feed on one C-resource only rather than simultaneously to both, since the preference is expressed toward the compound that returns the total higher microbial growth rate. The latter model will be regarded as model 2.

Both the formulations of β_i for model 1 and 2 consider implicitly that C-enzymes are inducible, the activation of which is determined by the presence of the substrate with a great return (Koch 1985) and that are not produced independently by the external conditions. Besides, it is assumed that in the window of observation *dt*, the daily scale, the gradual allocation of resources take place and thus microbes reorganize the production of enzymes to control substrates degradation and assimilation to match finally a condition that is the match law or the maximum return as microbial growth from the different C-sources. At the same temporal scale, microbes may express the growth rate in terms of generations, indicating that alternatively to the enzymatic regulation for a fixed population, we could assume in first approximation that the structure of community changes toward some species producing specifically a type of enzyme rather than growing on a single C, under the hypothesis that redundancy of functions exist.

The formulation for β_i assumes that the overlapping of resource consumption does not occur; on average and for each time step one every unit of biomass can thus complexate, feed and growth on a single chosen substrate only. In fact all the substrates are considered substitutable (homologous substrates after Koch 1985), hence C-compounds fulfil the same role in microbial metabolism neglecting any direct co-metabolic activity despite its importance within microbial community. However the formulation that considers the whole microbial community includes indirectly internal mechanisms since all the C flux from the both energetic and recalcitrant compounds contributes to the total return for the all microorganisms.

In both models the microbial distribution, as described by fractions β_i, is modeled in terms of microbial growth rates. I tried thus to simulate any type of factor that could influence the intake rates and regulate the microbial behaviour. For example the nutrient status such as the organic N present within the C pool or the mineral N amount available in the soil solution may be indicated as factors driving the microbial choice, markedly in combination of availability of labile C. Several hypotheses have been proposed, including the case of different microbial family growing on the several resources and competing for the same mineral N pool (Fontaine et al.2004; Fontaine and Barot, 2005) and the preferential substrate utilization (Cheng 1999; Craine et al. 2007; Blagodastakaya and Kuzyakov, 2008). The last mechanism has been supported by enzymatic studies that revealed the promotion of the C-enzymatic activity, while suppressing the oxidative activity under N fertilization (Sinsabaugh et al., 2002, 2005). Among these factors of biological nature, it has been considered the case of a changed decomposability of resources. For instance CO_2

fertilization may promote the production of C-rich litter. As well mineral N addition has been indicated as affecting the quality of SOM due to chemical reaction involving N-compounds and phenolic compounds. Temperature and soil moisture have been indicated affecting differently the decomposition of different C compounds (Henry et al.2005). Both the factors can be adduced to different microbial community or the promotion with temperature/moisture of a specific enzymatic expression (Dalias et al.2001; Henry et al. 2005). As it will be shown, the system behaviour is significantly different depending on the model used, in particular on the long term. In the first model, the nitrogen cycle has been added following the scheme reported in chapter 2 of the present work. In the second model the nitrogen cycle will not explicitly included, but the substrate preferential choice will simulated applying a reduction factors on the microbial growth rate per unit of microbial biomass. This reduction factor is applied to the growth rates for the determination of parameter β_i only, but it does not change the decomposition rates.

4.3 Qualitative study of the models

The set of ordinary differential equations was solved numerically using MATLAB (version 7.0.1) for different values of the model parameters, litter input or simulated external conditions allowing the system to run until a condition of equilibrium is achieved or for a long temporal scale. As a first attempt most of the parameters values were taken from literature, where available. The study was performed simulating the behaviour of a community B (g Cb m^{-2}) growing on two substrates, i.e. litter and SOM, thereinafter simply referred to C1 and C2 (g C m-2). I assigned to K_{s2} a value of 100 gC m^{-2} and K_2 a value of 0.0242 g C g Cb^{-1} m^{-2} d^{-1} (Moorhead and Sinsabaugh 2006). With regard to K_{a1} the value 6.5 10^{-5} g Cb^{-1} m^{-2} d^{-1} as estimated by D'Odorico (2003) was used as a first attempt; K_i was set as 0.01 d^{-1} (3-years turnover); Km was set arbitrarily as 30 g Cb^{-1} m^{-2}; microbial uptake of mineral N was set equal to 0.01 d^{-1}; parameter λ constitutes the contribution of microbial mortality and release of byproducts. Contrarily to microbial growth rate that is linked to C-uptake and hence to organic substances decomposition, estimation of λ is more challenging. In literature the equivalent to parameter λ ranges between 0.95d^{-1} for Blagodastky and Richter (1998) who tried to compute the mortality rate, 0.2 d^{-1} for labile part for Cochran (1988); 0.005 d^{-1} in Van Veen (1984) for recalcitrant part of microbial tissue; 0.001d^{-1} as estimated by Mueller (1996). I decided to keep fix the value of λ to 0.01 d^{-1}.External input was let range between the equivalents of 50-200 g C m^{-2} y^{-1}. Microbial yield was fixed to 0.45 for C1 and 0.5 for C2 in order to consider that SOM has more N and hence the efficiency increases with nutrient status (Manzoni et al., 2008).

4.4 Qualitative study: results and discussion

The present qualitative study is addressed to the observation of the simulated patterns of the two models under different working condition and the capability to mimic some experimental evidences, trying to overcome the difficulties induced by the presence of several populations.
It will be considered the case of changed litter input, a mechanism that can be induced by the CO_2 fertilization that promotes the photosynthetic activity and thus plant productivity, by shift of specie composition toward a vegetational composition that supply more carbon to soil via leaves and root turnover or by unfavourable climatic condition leading to a diminishing of litterfall production.
It will be investigated the pattern of variables on long term and the capability to mimic the priming effect. For instance, the basic model as it is formulated considering explicitly the microbial pool, should be able to reproduce the priming effect, a phenomenon referred to the case where the SOM mineralization is promoted, usually when labile C is added to the system (Blagodastakya and Kuzyakov 2008). In fact the increase of B promoted by litter input would supports a further decomposition of both substrates, C1 and C2, rising the number of decomposers feeding on resource C2. However it will be analysed the potential for simulating the negative priming, hence a mechanism inducing a retarding mineralization process. Besides it has been considered indirectly, as previously mentioned, different growing factors changing the intake rates and hence the microbial preferences.

4.4.1 MODEL 1

The decomposition model with linear dependence of mineralization of litter on microbial biomass allows easily the determination of the partitioning coefficients following the modified input matching law. In condition of constant litter input, on the long temporal scale, only the amount of biomass is correlated to the external input (it can be proved mathematically solving the equation systems under stationary condition). The C2 pool that constitutes the more recalcitrant pool and hence the soil C stock on the long term, is related to B *via* the parameter λ. On one hand this assures that once litterfall stops the soil C pool does not disappear but approach a limit value that depends on λ and the chemical characteristic of the C-compounds, hence the decomposition is not self-sustained but indirectly depends on the presence of biomass. On the other hand the increase of litterfall does not permit any accumulation of C2.
I let the system run to equilibrium for a litter input of 0.5 g C d^{-1} m^{-2} to achieve the initial condition B=45 g Cb m^{-2}, C1=340 g C m^{-2} and C2=480 g C m^{-2}. Litter input was then modified setting a value of 0.08 and 0.82 g C d^{-1} m^{-2} correspondent to an annual litterfall of 30 and 300 g C m^{-2} respectively. In this simple test, input of resources remains constant throughout simulation time and litter

changes are considered to occur abruptly from year 0 to the first year. Depending on the value of litter input the behaviour of the system on long time was observed, and thus the pattern of C1 and C2 was determined internally by the microbial choice and by chemical characteristics of substrates available in the system. The disturbance effects are shown in fig.2:

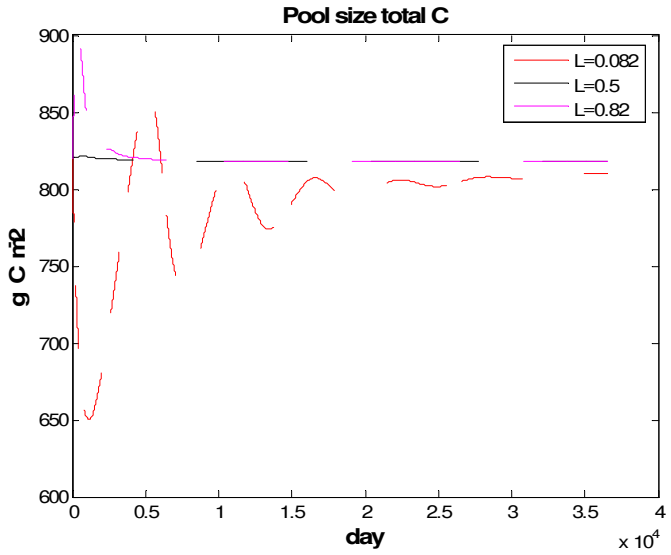

Fig. 2 Pattern of total soil C pool (C1+C2) for litter input L=0.082-0.5-0.82 g C m^{-2} d^{-1}.

Fig. 2 and Fig. 3 report the consumer distribution and the pattern of the C1 and C2 pool, namely litter and SOC pool in 100 year of simulation after the litter input has been changed from the situation of equilibrium. Since the resources are two, I simply reported one value of β, being the other one the complementary value. The tendency of the system is toward the condition where half of the biomass is on one pool and the other half on the other pool, for constant litter rate.

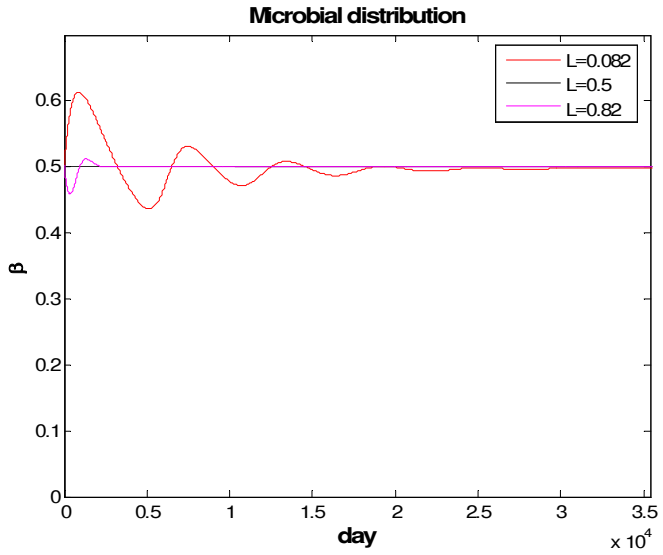

Fig. 3 Pattern of microbial distribution among the two resources C1 and C2.

The initial state and the amount of litter determine the pattern in time and the time for reaching the stationariness. The system tends to attain the previously achieved condition, since the microbial parameters were not modified, but with a different pattern. Markedly, the most important effects on the short term are expected to occur when considering the different CO_2 fluxes rather than the mineralized N fluxes when model is coupled to N cycle.

In a further case study, the effects of changed degradation rates of C1 rather than C2 pool were assessed. Litter input has been increased up to 0.8 g C m^{-2} d^{-1} from the case of reference L=0.5 g C m^{-2} d^{-1}. The long term C-stock of one pool behaves independently by the chemical characteristics of the other resource, thus affecting the decomposition of i.e. C1 pool, the C2 final size is not affected (Fig. 4 A-B). Analogously microbial pool B size depends by litter input only. A potential change of quality rather than accessibility of a specific resource influence the final pool of that resource only. Nevertheless, the pools size and its chemical characteristic influence the pattern of the system during transient conditions driving the partitioning of microorganism on the available resources.

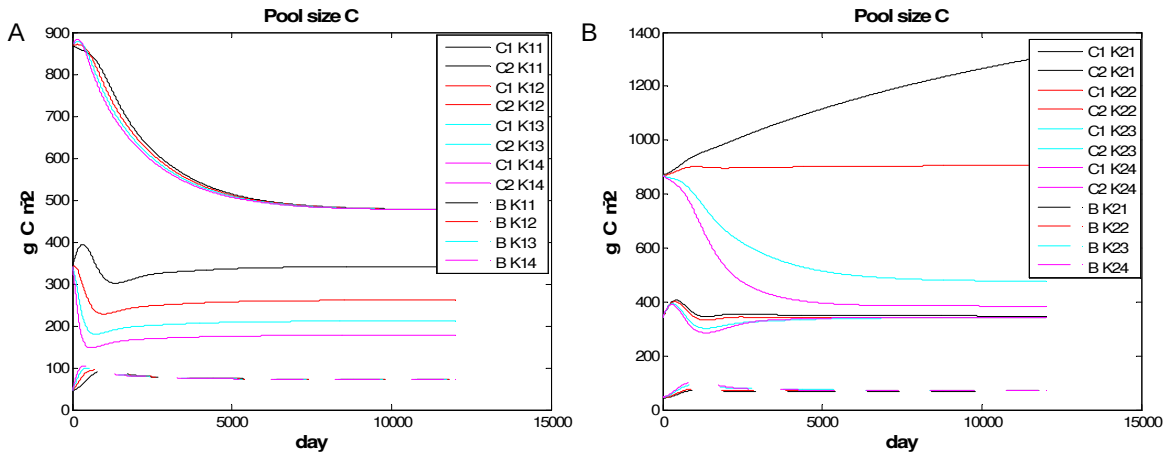

Fig. 4 Pattern in time of the C-pools C1, C2 and microbial pool B for a litter input L=0.8 g C m^{-2} $d^{-1}$and different values of decomposition constants. A) C1, C2 and B pools size under the conditions: K11=0.01, K12=0.009, K13=0.008, K14=0.007, K2=0.02 B) C1, C2 and B pools size under the conditions: K1=0.01, K21=0.02, K22=0.019, K23=0.018, K24=0.017; (note that the scales are different).

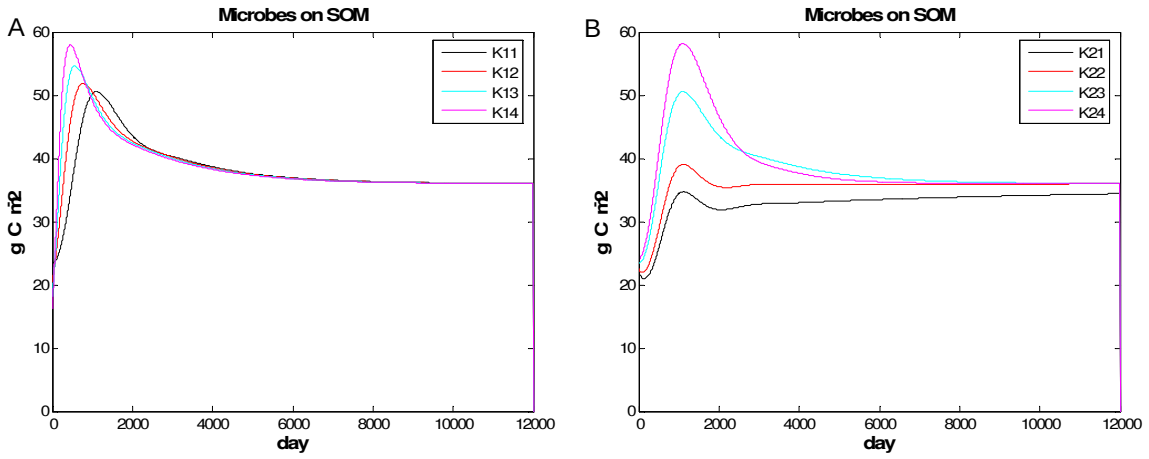

Fig. 5 Amount of microorganisms feeding on the C2 pool for a litter input L=0.8 g C m^{-2} $d^{-1}$and different values of decomposition constants. A) K11=0.01, K12=0.009, K13=0.008, K14=0.007, K2=0.02 B) K1=0.01, K21=0.02, K22=0.019, K23=0.018, K24=0.017.

However on the long term the microbial behaviour drives the system toward a condition where the amount of consumers feeding on C2 pool is the same (Fig. 5).

A further example in transient conditions, can be performed hypothesing the case where litter input stops and the system runs on the long term and the chemical characteristics of the different C-resources were changed. It is considered the effect of temperature as affecting positively the decomposition of C1 (T=T1,T2 with $K1<K1_{T1}<K1_{T2}$), the total final C is lower compared to the reference case as expected. However, compared to the previous case where C2 size is not affected by changed K1, in the transient condition the final C2 pool is lower as K1 increases (Fig. 6 A-B).

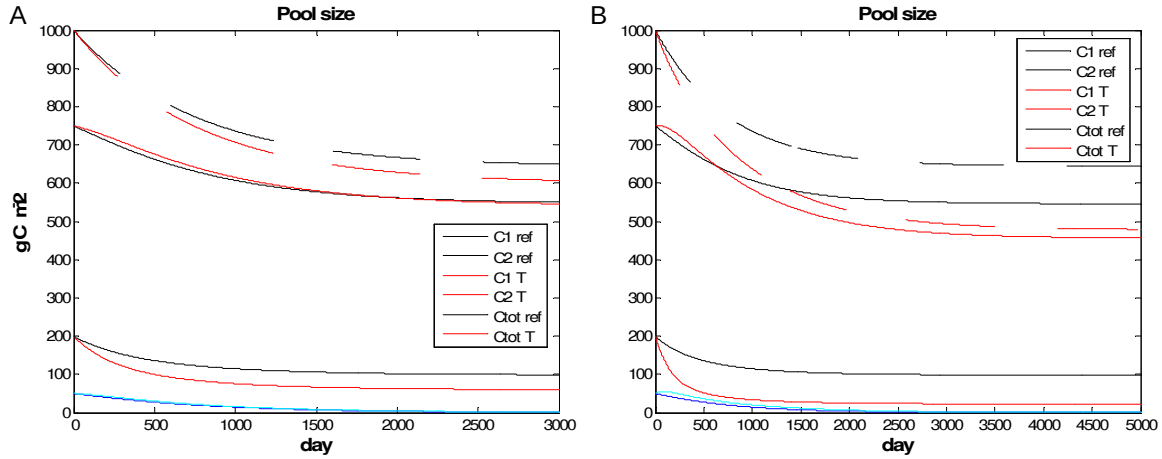

Fig. 6 C-Pools C1, C2 and B (blue lines), Ctot (C1+C2) in time (days). Ref is the situation of reference, K1=0.000065; A) T=T1, K1=0.00065; B) T=T2, K1=0.0015.

In a first phase, for the same % mass loss compared to reference case, more recalcitrant C has been left (Fig. 6 A-B) because the choice of microbes has moved weakly toward the C1 substances and away the native organic matter C2. If the value of K1 promotes a higher B growth, a significant

positive priming follows, that appears earlier for higher K1 values (Fig 6 B). Once the labile substrate is finished, i.e. due to the intermittence of input or when C1 is not profitable enough, all the microorganisms move again to degrade the native SOM. Since the amount of biomass is higher compared to what was the initial condition the positive priming occurs and in turn this leads to a final C2 pool lower as K1 increases. Normally the priming effect arises immediately or shortly after the addition of a specific substance to soil depending on the quality and on the amount of substrates added. The observed model behaviour has its correspondence in some experiments performed with labelled substrates or with the enzymatic pattern (Fontaine et al. 2004; Sinsabaugh et al., 2002, 2005; II chapter of this work). However, the time scales of occurrence of the priming effect is different from the lab experiment, for instance the differences are mainly related to two reasons: the nature of labile substrate, usually glucose or cellulose that are quickly assimilated promoting to the higher biomass in few days. Besides, the present model does not consider that a unit of biomass may feed on either the resources or that a changed microbial assimilation efficiency may occur.

The final remaining C pool seems to have a higher % of recalcitrant C with increasing T effect. The unchanged temperature sensitivity of pool C2 may have contributed as well to the observed pattern. The model results may for example explain the results reported in laboratory under different T condition by Dalias and coauthors (2001). It was found that at the same percentage of mass loss, the recalcitrant C was higher in the samples incubated at higher temperatures while more labile C has been left at lower incubation temperature, thus this could be justify by the preferential choice for labile C leaving more recalcitrant C. Hence the different quality of resources or the different capability to degrade C1 respect to C2 may affect the microbial choice as showed by the modelling approach. However it is not possible to sustain if longer in the experiment, more C was finally released as in our case study.

Model 1: Nitrogen limitation

As mentioned previously, the linear model does not allow either for the accumulation of C when litter input to the system increase or the accumulation of C for a constant input litter. Such goal may be achieved when including the N cycle, for instance the N limitation implies that C may accumulate in the system because of a reduction of C degradation (Molina et al.1983). Fig. 7 shows the pattern of soil C pools for three different litter input rates (conditions: C/N litter= 200, C/N soil=30, C/N biomass=8), and the same level of inorganic N=0.85 g N m^{-2}, starting from an arbitrary initial condition. For the highest litter input, total C increased in time due to a lower decomposition induced by a lower presence of mineral N that does not satisfy the microbial demand. On the long time C1 and C2 pools sizes do not depend on litter input when N does not limit microbial growth, hence in the case of study for L=0,25 and 0.5 g Cm^{-2} d^{-1}. It is notably how

the system is really sensitive to the N limitation and the C pool increases abruptly once the microbial activity is reduced.

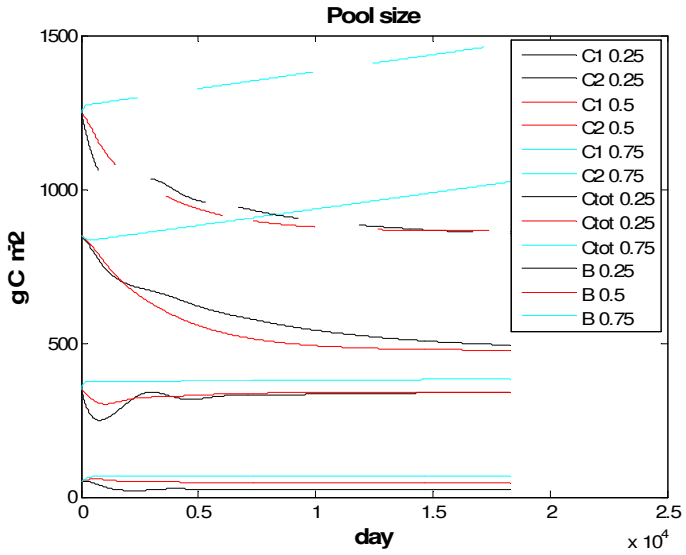

Fig. 7 Pattern in time of the C-pools C1, C2, Ctot (C1+C2) and B for different input value; L=0.25-0.5-0.75 g Cm^{-2} d^{-1}, with a constant mineral N=0.85 g N m^{-2}.;

Analogously a further qualitative study has been conducted keeping fixed the litter input L==0.75 g C m^{-2} d^{-1} and mineral N availability has been set to N=0.85-0.87-1 g N m^{-2}. As expected, C pools decreased drastically once the mineral N pool increased (Fig. 8), in particular the system is sensitive when is close to condition of N-limitation (see the pattern from N=0.85 g N m^{-2} to N=87 g N m^{-2}).

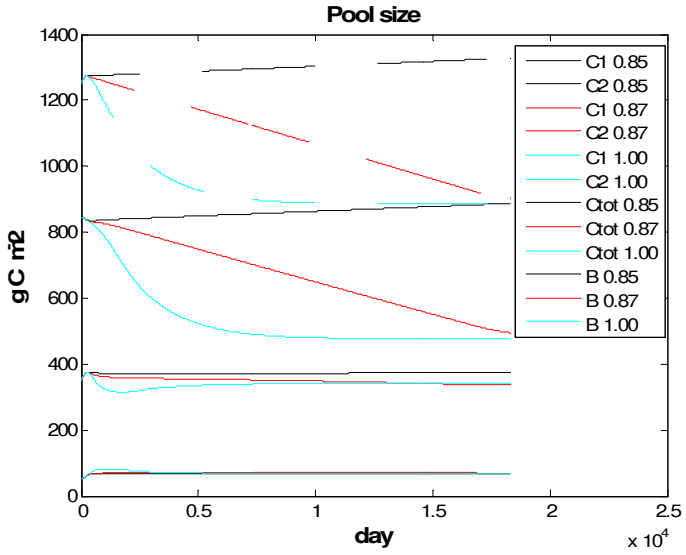

Fig. 8 Pattern in time of the C-pools C1, C2, Ctot (C1+C2) and microbial biomass B for different value of soil mineral N; N=0.85, 0.87,1 g N m^{-2} and a constant input of litter L=0.75 g Cm^{-2} d^{-1}.

The showed simulation results would state that once litter production increases concurrently with mineral N, a trend toward a low C stock would occur. However, this result is in contrast with experimental evidences that suggests, at least in forest ecosystems that increasing N availability (i.e.

due to wet and dry N deposition) promotes a trends toward a soil becoming a C-sink (Reay et al. 2008). Besides, a meta-analyses by van Groeningen (2006) reported the positive effect of CO_2 fertilization effect among N fertilization, on soil C stock (Fig. 9), supposing that increasing N fertilization rates implies an increase of soil N availability.

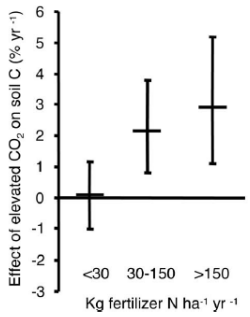

Fig.9 The effect of elevated CO_2 on soil C contents. Changes in soil C contents as affected by N fertilization. There is a significant difference between N fertilization classes (P<0.02). The values for 30, 30–150, and 150 kg ha^{-1} yr^{-1} of N are based on 43, 25, and 12 observations, respectively. (after van Groeningen et al.2006).

This trend is the results of the N effects at level of plant and microbial community. With regards to the first item N may affect litter production and quality, while affecting microbial efficiency and degradation rate due to change substrate quality (Franklyn et al.2003; Agreen and Bosatta 2001). However this is the results of a model fit to experimental data. Thus, what is interpreted by model as a reduction of SOM decomposition could be indeed the result of the shift away from native SOM and the increasing assimilation to degradation C, with the net effect of a lower C-loss from the system.

Thus, a more reasonable model result can be achieved if the litter input increases with rising N availability, is coupled to a change of substrate preference. In general terms, the microbial choice may be modified because the specific pool of interest has a low rather than high organic N content or because the availability of labile N, i.e. mineral N, supports the use of labile C. In these conditions the preference is moved toward the most suitable resource that allows the growth with the lower N limitation.

The preference for the substrate is not due to the changed quality of C-compounds hence the modification of the potential intake rates is applied in the formulation for β only, in order to change the number of decomposers feeding on the specific C-resources. In this way the system does not achieve a state where half of decomposers growth on a resources and the other half on the other one, but a new equilibrium is achieved, where the preference of microbial biomass is significantly altered, but not the degradation rate per unit of microbial biomass.

As a first attempt I do not explicit the final substrate choice as direct function of mineral N or C/N ratio of C-pools but I simply modified the potential intake rates using a reduction factor ranging between 0 and 1.

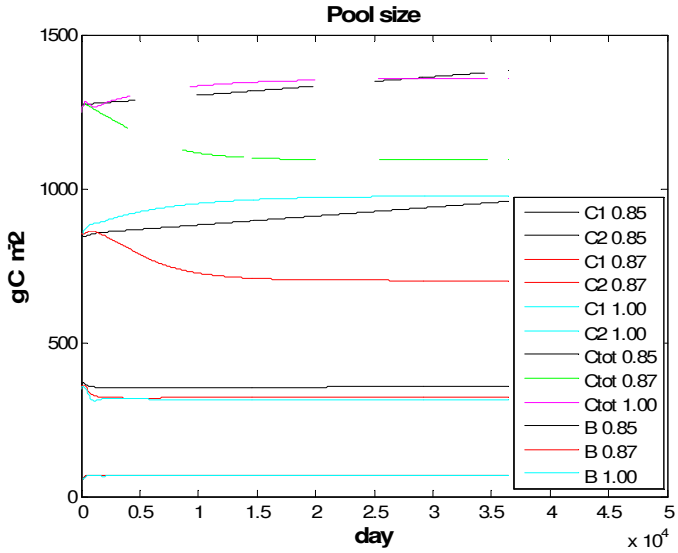

Fig. 10 Pattern in time of the C-pools C1, C2, Ctot (C1+C2) and microbial biomass B for different value of soil mineral N; N=0.85, 0.87,1 g N m^{-2} and a constant input of litter L=0.75 g Cm^{-2} d^{-1}, with a lower preference for C2 pool decreasing with increasing mineral N pool (see text).

Fig.10 shows the case study where the intake rates from the C2 pool were modified and the microbial choice toward the C2-compounds was diminished. Obviously the same qualitative behaviour holds for the case in which the modification potential gain regards the C1 pools although quantitatively is different. The present case can be referred to the negative priming due to the lower preferences for the more recalcitrant pool, for fixed litter input (Blagodastakya and Kuzyakov 2008). However the behaviour does not follow a positive trend according to the amount of mineral N, since for the same litter input the maximum C pool on the long term is not achieved under the condition of maximum inorganic N availability. For instance, the microbial preferences have been kept fixed in time while the preference could change in time depending on the microbial-C request. At the present, I did not consider any change of microbial efficiency in the presence of higher nutrient status as would be suggested by the experiment performed by Fontaine (2005) supplying labelled cellulose and different amount of mineral N. This in turn would support a higher C retention in the system.

4.4.2 MODEL 2

In the next case study I used the second model where the non linear dependence of litter decomposition on microbial biomass is expressed to let the C pools be dependent on litter input. In this case I applied the condition that the goal of the microbial biomass is the maximization of the

microbial growth rate at each time step of observation, considering the contribution to growth from different C-sources. Compared to the previous model nitrogen cycles has not been explicitly included.

Starting from the same initial condition I let the system reach the equilibrium condition for different litter input. The microbial distribution oscillates around the value 0.5 but without approaching that value as in the case of matching law in Fig. 3 (data not shown). Compared to the model 1, C1 and B pools increased under increasing litter input while the opposite occurred for C2 pool (data not shown) and the final result exhibits a lower total C as litter input increases (Fig. 11).

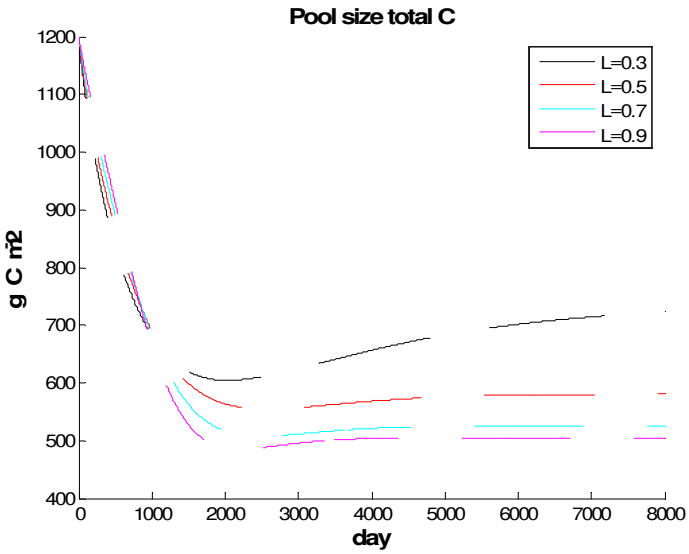

Fig. 11 Pattern of total C pool (C1+C2) for different input of litter; L=0.3-0.5-0.7-0.9 g C m^{-2} d^{-1}.

In fact, despite C2 pool depends on B amount via the parameter λ that controls the production of recalcitrant compounds (equation not presented), concurrently a positive priming was exhibited (Fig.12). The amount of microbes feeding on C2 is higher in the case with increasing litter input to the system. This simple model experiment would support hence the presence of a priming effect that may work on yearly time scale (Carney et al. 2007) and not only at the daily time scales (Fontaine et al.2004). The observed pattern have been investigated analyzing the enzymatic activity in the work of Carney and co-authors (2007), where a higher oxidative activity (devoted to degradation of recalcitrant compounds) was observed.

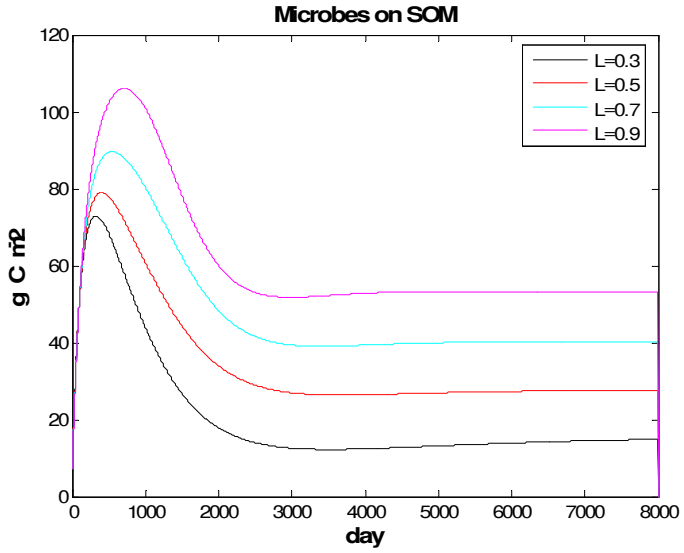

Fig. 12 Amount of microorganisms feeding in time on C2 pool (SOM) for different input of litter; L=0.3-0.5-0.7-0.9 g C m^{-2} d^{-1}.

I let the system run to reach the stationary condition under litter input L=0.5 g C m^{-2} d^{-1}, K2=0.02, K1=0.01, Ks=100, Km=30 as fixed arbitrarily. For final values of C1=135 g C m^{-2}, C2=445 g C m^{-2}, B=45 g C m^{-2}. A case study was performed increasing litter input from this condition of equilibrium. I performed the simulation along 50 years with an initial abrupt increase of litter input. Fig. 13 shows the pattern of the different soil C pools; after an initial period of promotion of increase of SOM pool due to the higher microbial preference toward C1 pool, the diminishing of C2 resource is the direct consequence of the positive priming effect, as highlighted in the previous case study. Compared to the previous model, the behaviour for different litter input is smoother and the oscillations of microbial community are less evident.

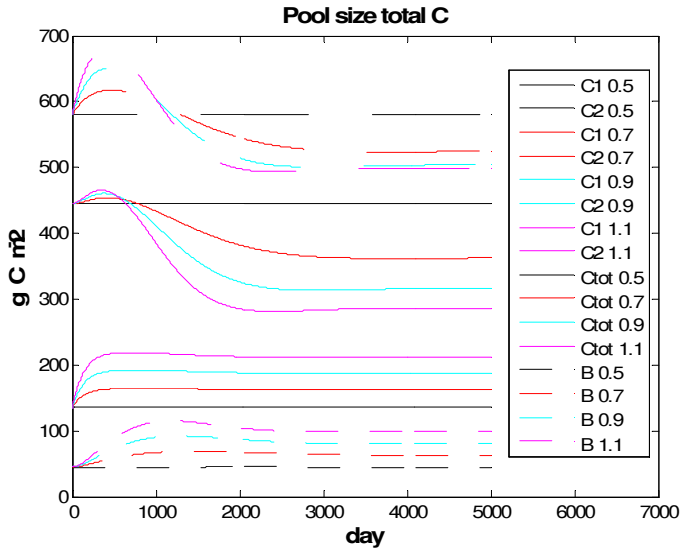

Fig. 13 Pattern in time of the C-pools C1, C2, Ctot (C1+C2) and microbial biomass B for different value of litter input L; L=0.5-0.7-0.9-1.1 g C m^{-2} d^{-1}; Ctot, sum of C1 and C2; B, is the microbial biomass pool.

Analogously to the model 1, it has been performed a simulation where the preferences for a specific resources has been modified using a factor that changes the microbial choice, but without altering the degradation rate per unit of microbial biomass. This factor is not explicit function of C/N ratio of the several resources or mineral N availability, but affects the potential intake rates only. The preference for C2 has been diminished to consider how the presence of mineral N may shift the preference toward the more labile-C (Fig. 14). Markedly, the preference for C2 has been diminished as litter input increased hypothesizing a concurrent increase of mineral N.

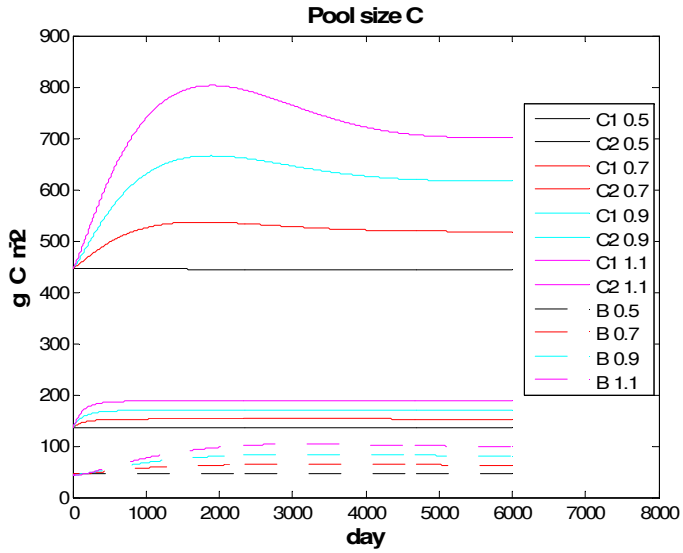

Fig. 14 Pattern in time of the C-pools C1, C2, Ctot (C1+C2) and microbial biomass B for different value of litter input L; L=0.5-0.7-0.9-1.1 g C m^{-2} d^{-1}; Ctot, sum of C1 and C2; B, is the microbial biomass pool. Preference for pool C2 has been changed i.e. decreased, to mimic the more suitability of C1 pool.

Fig 15 B shows as the amount of microorganisms growing on C2 pool increase as litter input increases as expected, but for fixed litter input the amount of microbes decreased if compared to the previous case where the preferences has not been modified (Fig. 15 A); microbial biomass is dependent on the litter input only and not on the microbial choice (data not shown). When considering the C stock, the negative priming occurred due to the changed preference. In fact the positive priming is induced by the higher amount of litter, however, for the same amount of external input, the system has moved toward a condition of higher C tot (data not shown) due to the decrease of amount of microbes feeding on C2 pool. The same qualitative behaviour has been reported in the experiment of Fontaine et al (2004), where a soil incubation has been carried adding the amount of labelled cellulose (the equivalent of C1 pool) at the beginning of the incubation and for different amount of soil mineral N. In the case with both C1 and N fertilization, the total amount of C left in the soil at the end of incubation was higher compared to the reference case. The accumulation of soil organic carbon under increasing atmospheric CO_2 and N fertilization, showed by van Groeningen (2006), could be effectively the result of a strategy that lead microbial community to a

high efficiency condition and a lower loss of C from the system in the presence of labile C. However, the presented models do not contemplate the toxic effect induced by the presence of surplus of mineral N in particular in form of nitrate. Besides, it is important to keep in mind that most of the experiment performed in the field deal with an abrupt rise of CO_2 and N in the environment, while it seems that for gradually increase of external condition the change of microorganisms could be more smooth (Klironomos et al.2005) and the temporal scale of adaptation different.

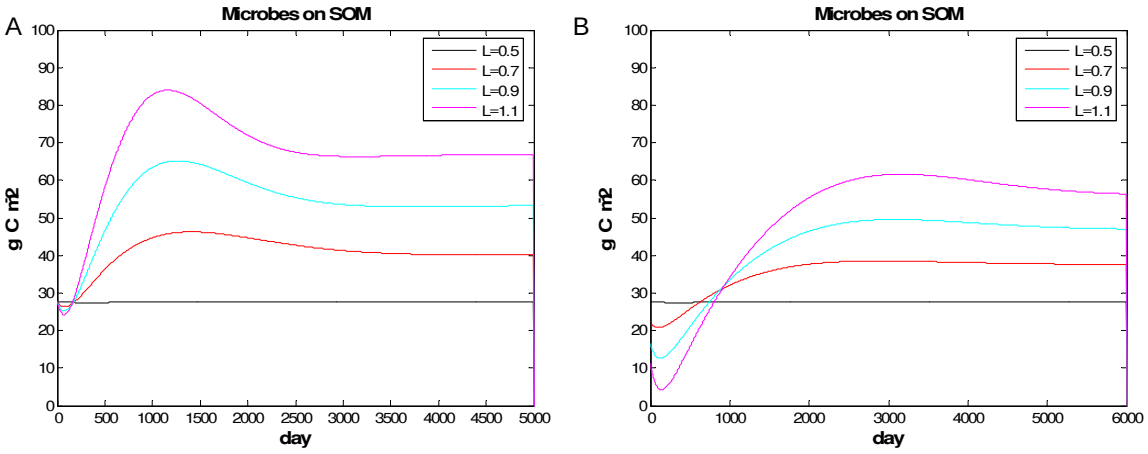

Fig. 15 Amount of microorganisms feeding in time on C2 pool (SOM) for different input of litter; L=0.3-0.5-0.7-0.9 g C m^{-2} d^{-1} A) preferences for C2 pools determined by C sizes pools and chemical characteristics, B) case with decreasing preferences for resource C2.

In the following simulation the preference for C1 pool has been changed to mimic a lower preference due to the low amount of organic N, for example a situation that reflect the production of C-rich litter under condition of CO_2 fertilization. In this case the microbial choice is toward the resource that can guarantee a sufficient amount of N for microbial growth, as it is shown in fig.15 where a higher amount of biomass is decomposing C2. For instance microbes perceive the high availability of labile C but they decompose SOM to gain N. The mentioned mechanisms has been indicated as mining (Craine et al. 2007) and refers to the case where N limitation address the decomposition of native SOM to obtain N.

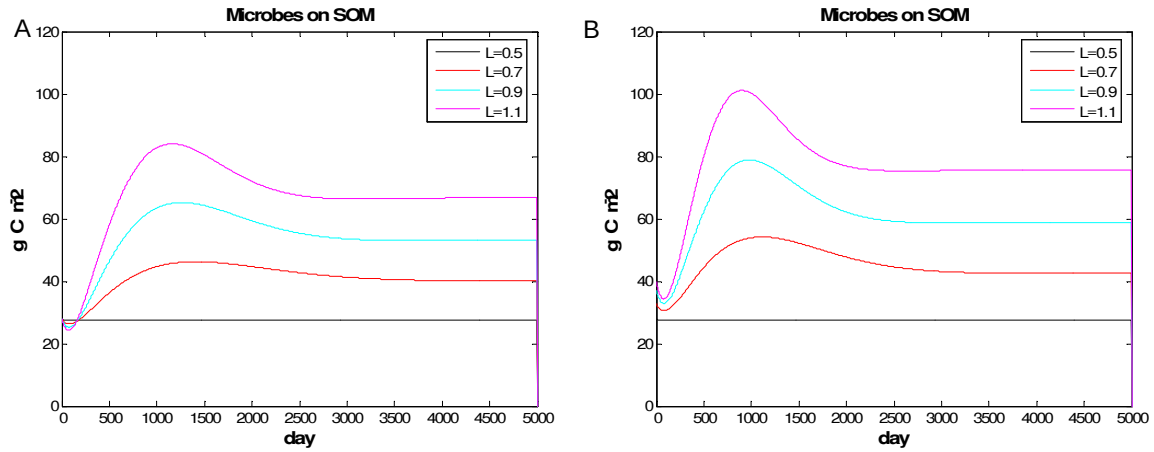

Fig. 16 Amount of microorganisms feeding in time on C2 pool (SOM) for different input of litter; L=0.3-0.5-0.7-0.9 g C m^{-2} d^{-1} A) preferences for C2 pools determined by C sizes pools and chemical characteristics, B) case with increasing preferences for resource C2.

Analogously to model 1, litter input has then been increased up to 0.8 g C m^{-2} d^{-1} from the case of reference L=0.5 g C m^{-2} d^{-1} and in a second case, has been kept as zero, but the degradation constant K1 and K2 have been changed. The exhibited pattern is similar to the one observed for the model 1 (Fig. 17-18-19). On the long temporal scales both B and the pool were not affected by differences in K, but are independent by the changed characteristics of the other pool. It means that after a first period, microbes moves the resources toward a condition where the number of microorganism feeding on C2 remains the same, but the lower decomposition rates allows for accumulation of total soil C.

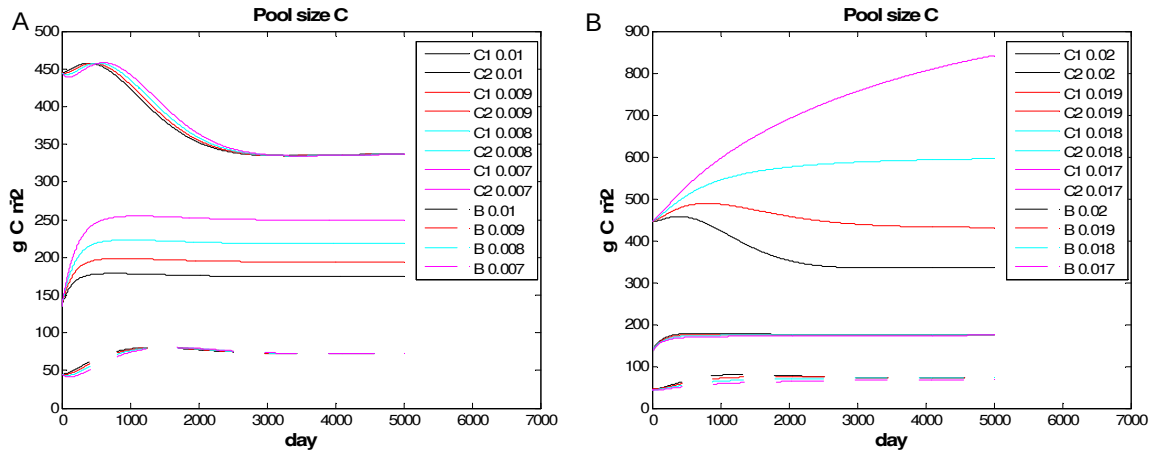

Fig. 17 Pattern in time of the C-pools C1, C2, Ctot (C1+C2) and microbial biomass B for a litter input L=0.8 g C m^{-2} $d^{-1}$and different values of decomposition constants. A) K1=0.01-0.009-0.008-0.007, K2=0.02 B) K1=0.01, K2=0.02-0.019-0.018-0.017; Ctot, sum of C1 and C2; B, is the microbial biomass pool (note that the scales are different).

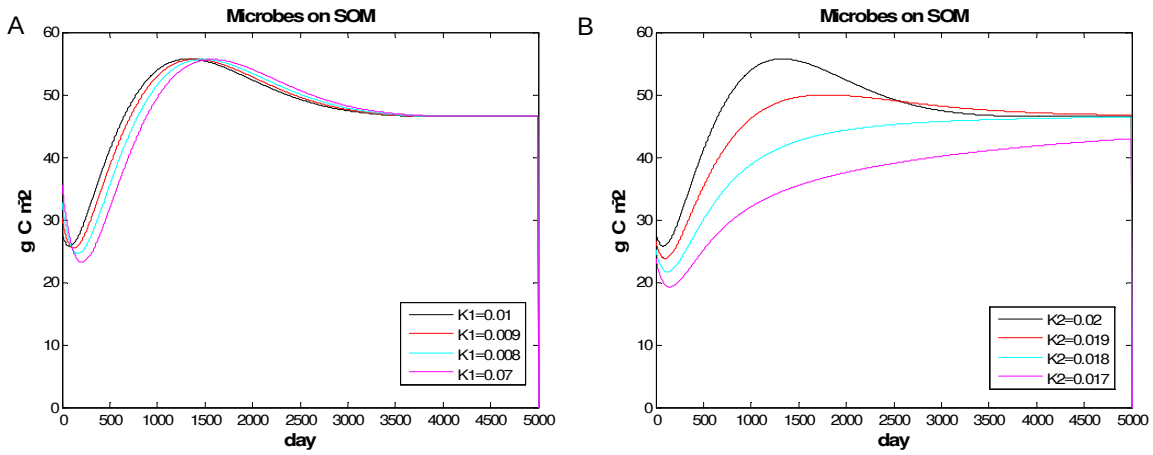

Fig. 18 Amount of microorganisms feeding on the C2 pool for a litter input L=0.8 g C m^{-2} $d^{-1}$and different values of decomposition constants. A) K1=0.01-0.009-0.008-0.007, K2=0.02 B) K1=0.01, K2=0.02-0.019-0.018-0.017.d^{-1}.

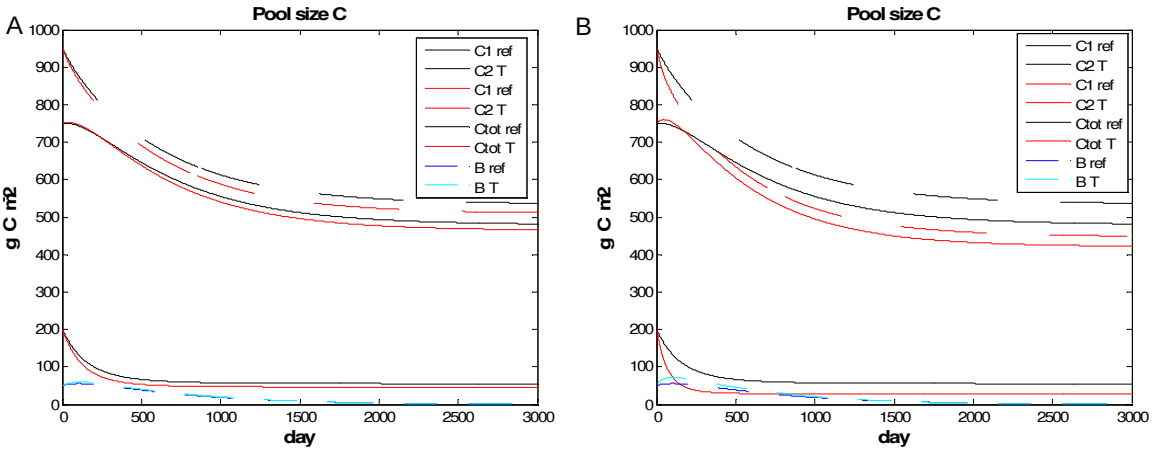

Fig. 19 C-Pools C1, C2, Ctot (C1+C2) and microbial biomass B in time (days). Ref is the situation of reference, K1=0.01; A) T=T1, K1=0.012; B) T=T2, K1=0.02.

Compared to model 1 that assures that there is always a small amount of biomass feeding and growing on the resources, the second model may return a pattern where the preferential utilization of one substrate only may occur depending on the amount of resources and the quality (data not shown). Such condition would imply that microbes would feed on litter only that is actually the most suitable substrates when nutritional status supports the choice toward litter or the more labile C compounds. Thus a further criterion is needed to guarantee that at least a small amount of microorganism growth on the less favourable resource. However the ecological assumption behind the model 2 appears the most robust scheme.

The next step will include the study on long term for different values of λ, microbial yields and microbial choice parameters as expression of C/N of compounds and mineral N. In fact at the current state I used fixed and constant microbial yield, whilst it has been shown both theoretically

and experimentally how microorganisms may increase their assimilation efficiency in condition of a more favourable N status (Manzoni et al.2008; Fontaine et al., 2004, Agreen and Bosatta 2001).

4.5 Conclusions

The present simple models were the first attempt to include in soil model some formulations of substrate preference and co-limitation of decomposition by both substrate and decomposers with regards to degradation of soil organic matter within the contest of soil C-cycle. The qualitative observed pattern would make in discussion the condition of equilibrium in forest ecosystems but would support again how climate change and N deposition may alter the microbial activity and the process of soil C sequestration based on mechanisms of ecological nature. The complete mathematical formulation is similar to a population model studying the behaviour of a consumer-resources system. Markedly it has been referred to the input matching law and the achievement of an ecological goal considering the microbial community as a single individual working for the optimization of the total microbial performances (i.e. growth). Among optimization procedures used for plant C-allocation in some recent studies in literature, the same ecological concept applied to soil biological behaviour in the second model, constitutes a promising tool in order to deal with the complexity of mechanism that are present in the soil and to better understand the interactions of soil biology with abiotic factors. Although the simplicity of the second model and the several critical points that can be moved toward the choice of optimality criterion and microbial working condition, it is suggested how the consideration of the maximization of a specific ecological goal may be useful to explain some experimental evidences in literature at macroscopic level. Namely the positive and negative priming, and the potential for C accumulation in the presence of mineral N. Moreover it was an attempt to use external variables such as litter production, pools size and substrate quality to mimic the population behaviour without entering in the complexity of several populations existing and interacting for substrates (i.e. competition, co-metabolism etc). Experimentally, the model suggests how the enzymatic fingerprint of microbial community could be assessed as the link between the microbial community, considered as a whole feeding on the mixed substrates, and the ecological function expressed, thus the organic matter degradation.
The future proposing work regards mainly a deeper qualitative study of the model behaviour (i.e. different functions describing the input of resources in the system), evaluating the behaviour of the model 1 with maximization growth and model 2 with the application of the matching law. Besides the expression of reduction factors toward microbial choice as explicit function of soil N status will be considered.

Reference

Ågreen, G.I., Bosatta, E., 2001. Combining theory and experiment to understand effects of inorganic nitrogen on litter decomposition. Oecologia 128, 94–98.

Allison, S.D., Vitousek, P.M., 2005. Responses of extracellular enzymes to simple and complex nutrient inputs. Soil Biology & Biochemistry 37, 937–944.

Bahn M., Knapp M., Garajova Z., Pfahringer N., 2006. Root respiration in temperate mountain grasslands differing in land use. Glob Change Biol 12, 995-1006.

Berg, B., McClaugherty, C., Johansson, M-B., 1993. Litter mass-loss in late stages of decomposition at some climatically and nutritionally different pine sites. Long-term decomposition in a Scots pine forest. VIII. Canadian Journal of Botany 71, 680±692.

Blagodatsky, S.A., Yevdokimov, I.V., Larionova, A.A., Richter, J., 1998. Microbial growth in soil and N turnover: model calibration with laboratory data. Soil Biology & Biochemistry 30, 1757-1764.

Blagodatsky, S.A., Richter, O., 1998. Microbial growth in soil and nitrogen turnover: a theoretical model considerino the activity state of microorganisms. Soil Biology & Biochemistry 30, 1743-1755.

Brandt, B.W., van Leeuwen, I.M.M., Kooijman, S.A.L.M., 2003. A general model for multiple substrate degradation. Application to co-metabolism of structurally non-analogous compounds. Water research 37, 4843-4854.

Burns, R.G., 1982. Enzyme activity in soil: location and a possible role in microbial ecology. Soil Biology & Biochemistry 14, 423–427.

Carney, K.M., Hungate, B.A., Drake, B.G., Megonigal, J.P., Altered soil microbial community at elevated CO_2 leads to loss of soil carbon. PNAS 12, 4990–4995

Chen, W., 1999. Rizhosphere feedbacks in elevated CO_2. Tree Physiology 19, 313-320-.

Cherif, M., Loreau, M., 2007. Stoichiometric constraints on resources use, competitive interactions, and elemental cycling in microbial decomposers. The American Naturalist 169, 709-724.

Cochran, V.L., Horton, K.A., Cole, C.V., 1988. An estimation of microbial death rate and limitations of N or C during wheat straw decomposition. Soil Biology & Biochemistry 20, 293–298.

Craine, J.M., Morrow, C., Fierer, N., 2007. Microbial nitrogen limitation increases decomposition. Ecology 88, 2105-2113.

Dalias, P., Anderson, J.M., Bottner, P., Coûteaux, M.M., 2001. Long-term effects of temperature on carbon mineralisation processes. Soil Biology & Biochemistry 33, 1049-1057.

Dewar, R.C., Franklin, O., Makela, A., McMurtrie, R.E., Valetnine, H.T., 2009. Optimal function Explains forest responses to global Change. Bioscience 59, 127-139.

P. D'Odorico, P., Laio, F., Porporato, A., Rodriguez-Iturbe, I., 2003. Hydrologic controls on soil carbon and nitrogen cycles.II. A case study. Advances in Water Resources 26, 59–70.

Ettema, C.H., Wardle, D.A., 2002. Spatial soil ecology. TRENDS in Ecology & Evolution 17, 177-183.

Fontaine, S., Mariotti, A.,, Abbadie, L., 2003. The priming effect of organic matter: a question of microbial competition? Soil Biology & Biochemistry 35, 837–843.

Fontaine, S., Bardoux, G., Abbadie, L., Mariotti, A., 2004. Carbon input to soil may decrease soil carbon content. Ecology Letters 7, 314–320.

Fontaine, S., Barot, S. 2005. Size and functional diversity of microbe populations control plant persistence and long-term soil carbon accumulation. Ecology Letters 8, 1075–1087.

Franklin, O., Högberg, P., Ekblad, A., Göran, Ågren, G.I., 2003. Pine Forest Floor Carbon Accumulation in Response to N and PK Additions: Bomb ^{14}C Modelling and Respiration Studies. Ecosystems 2003 6, 644–658.

Franklin, O., 2007. Optimal nitrogen allocation controls tree responses to elevated CO_2. New Phytologist 174, 811-822.

Givnish, T.J., (ed.). 1986. On the economy of plant form and function. Cambridge Univ. Press: Cambridge.

van Groeningen, K., Six, J., Hungate, B.A., de Graaf, M.A., van Breemen, N., van Kessel C., 2006. Element interactions limit soil carbon storage. PNAS 103, 6571–6574.

Henry, H.A.L., Juarezw, J.D., F i e ld, C.B., Vitousek, P., 2005. Interactive effects of elevated CO2, N deposition and climate change on extracellular enzyme activity and soil density fractionation in a California annual grassland. Global Change Biology 11, 1808–1815.

Jessup, C.M., Kassen, R., Forde, S.E., Kerr, B., Buckling, A., Rainey, P.B., Bohannan, B.J.M., 2004. Big questions, small worlds: microbial model systems in ecology Trends in Ecology and Evolution 19, 189-197.

Klironomos, J.N., Allen, M.F., Rillig, M.C., Piotrowski, J., Makvandi-Nejad, S., Wolfe, B.E., Powell, J.R., 2005. Abrupt rise in atmospheric CO_2 overestimates community response in a model plant–soil system Nature 433, 621-624.

Koch, A.L., 2005. Bacterial Choices for the consumption of multiple resources for current and future needs. Microbial Ecology, 49. 183-197.

Lessels, C.M., 1995. Putting re source dynamics into continuous input ideal free distribution models. Animal Behaviour 49, 487-494.

Manzoni, S., Jackson, R.B., Trofymow, J.A., Porporato, A., 2008. The Global Stoichiometry of Litter Nitrogen Mineralization. Science 321, 684-686.

Molina, J.A.E., Clapp, C.E., Shaffer, M.J., Chichester, F.W., Larson, W.E., 1983. NCSOIL, a model of nitrogen and carbon transformation in soil: description, calibration and behaviour. Soil Sci. Soc. Am. J. 47, 85-91.

Moorhead, D.L., Sinsabaugh, R.L., 2000. Simulated patterns of litter decay predict patterns of extracellular enzyme activities. Applied Soil Ecology 14, 71-79.

Moorhead, D.L., Sinsabaugh, R.L., 2006. A theoretical model of litter decay and microbial interaction. Ecological Monographs 76, 151–174.

Mueller, T., Jensen, L.S., Hansen, S. & Nielsen, N.E. (1996) Simulating soil carbon and nitrogen dynamics with the soil–plant–atmosphere system model DAISY. Evaluation of Soil Organic Matter

Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G., Renella, G., 2003. Microbial diversity and soil functions. European Journal of Soil Science 54, 655–670.

Panikov, N.S., 1999. Understanding and prediction of soil microbial community dynamics under global change. Applied Soil Ecology 11, 161-176.

Reay, D.S., Dentener, F., Smith, P., Grace, J., Feeley, R.A., 2008. Global nitrogen deposition and carbon sink. Nature Geoscience 1, 430-437.

Schimel, J.P., Weintraub, M.N., 2003. The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biology & Biochemistry 35, 549–563.

Sinsabaugh, R.L., Moorhead, D.L., 1994. Resources allocation to extracellular enzyme production: a model for nitrogen and phosphorus control of litter decomposition. Soil Biology & Biochemistry 26, 1305-1311.

Sinsabaugh, R.L., Carreiro, M.M., Repert, D.A., 2002. Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. Biogeochemistry 60, 1–24.

Sinsabaugh, R.L., Gallo, M.E., Lauber, C., Waldrop, M.P., Zak, D.R., 2005. Extracellular enzyme activities and soil organic matter dynamics for northern hardwood forests receiving simulated nitrogen deposition. Biogeochemistry, 75: 201–215.

Tregenza, T., 1994. Common misconceptions in applying the ideal free distribution. Animal Behaviour, 47, 485-487.

Trumbore S.E., Bonani G., Wolfi W., 1990. The rates of C cycling in several soils from AMS 14C measurements of fractionated soil organic matter. In: Bouwman A.F. (Ed.) Soil and Greenhouse Effects. Wiley, New York, pp. 405-414.

Young, I.M., Crawford, J.W., 2004. Interactions and Self-Organization in the Soil-Microbe Complex. Science 304, 1634-1637.

Van Veen, J.A., Ladd, J.N., Frissel, M.J., 1984. Modelling C and N turnover through the microbial biomass in soil. Plant Soil 76, 257–274.

Varner, J., Ramkrishna, D., 1999. The non-linera analysis of cybernetic models. Guidelines for model formulation. Journal of Biotechnology 71, 67-104.

Venkatesh, K.V., Doshi, P., Rengaswamy, R., 1997. An optimal strategy to model microbial growth in a multiple substrate environment. Biotechnology and bioengineering 56, 635-644.

Wutzler, T., Reichstein, M., 2008. Colimitation of decomposition by substrate and decomposers – a comparison of model formulations. Biogeosciences Discuss 5, 163–190.

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