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Abstract: Conservation agriculture represents an approach to managing agro-ecosystems aiming at preserving and enhancing the resource base and the environment. Subsidiary crops (SCs) are important components of conservation agriculture since they provide agro-ecological services, such as maintaining the soil resource. However, the importance of SC species and environment for the effect on soil microbial community are not well known. The overall objective of this study was to assess the effect of various subsidiary crops on soil microbial biomass and activity at four sites across Europe. The experiments were conducted during 2014 and 2015 at sites in the Nemoral (Sweden SLU), Oceanic (United Kingdom ORC), Continental (Switzerland AGS) and Mediterranean north (Italy UNI) pedo-climatic zones. The aims were to determine: (i) the effect of SC growth on soil microbial biomass and its activity, ii) the overall site-specific effect of the SCs growth of on soil biochemical properties. The SCs consisted of leguminous or brassicaceous species sown after harvest of wheat, or clover species under-sown in wheat. Microbial carbon and nitrogen quotients increased under SCs at the majority of sites indicating carbon and nitrogen immobilization process which may result in a positive trend of soil organic matter over time. Effects of SCs were similar in the pedo-climatic zones where temperatures are never below 0 °C (ORC and UNI). Moreover, arylsulphatase was the most sensitive enzyme to legumes in the Mediterranean north. In contrast, chitinase activity was enhanced by SCs in the Oceanic and Nemoral pedo-climatic zones. The high precipitation level and the low average temperature, typical of Continental and Nemoral zones, may represent limiting factors for soil enzyme activity under all selected SCs. Among the four pedo-climatic zones, the Mediterranean north represented the most suitable environment to promote SC growth and soil coverage. This study showed that SC cultivation affects soil quality enhancing biochemical activity; however this effect may be modulated by the different pedo-climatic conditions.

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Viterbo July 27th, 2017

To the Editor of
Soil Tillage Research

Dear Editor,

We wish to submit an original research article entitled "**Changes of soil biochemical properties as affected by subsidiary crop cultivation in four European pedo-climatic zones"** for consideration by Soil Tillage Research Journal.

We confirm that this work is original and has not been published elsewhere, nor is it currently under consideration for publication elsewhere.

In this paper, we discuss the effect of various subsidiary crops cultivation on soil microbial biomass and activity at four sites across Europe. Among the four pedo-climatic zones, the growth of legumes as living mulch enhances soil specific enzyme activity producing similar effects in pedo-climatic zones where the mean temperatures do not fall below 0 °C (Mediterranean north and Oceanic, respectively). Moreover, the Mediterranean north is the most suitable to promote growth and coverage of leguminous SCs, enhancing soil microbial activity and functional diversity. As for Continental and Nemoral zones, the high precipitation level and the low average temperature, respectively may represent a limiting factor for soil enzyme activity under all selected SCs.
In this study, subsidiary crops utilization is confirmed to be an effective agricultural practice promoting soil biochemical properties even before their suppression. However, the positive effects may have different extent in relation to the specific pedo-climatic area.

We declare that we do not have conflicts of interest to disclose.
Please address all correspondence concerning this manuscript to me.
Thank you for your consideration of this manuscript.
Sincerely,

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Highlights

1. SC cultivation affects soil quality enhancing biochemical activity
2. SCs effect on soil biochemical properties were similar in the mild pedo-climatic zones
3. High rainfall and low temperature may reduce the effect of SCs on soil microbial activity.
4. The Mediterranean north was the most suitable climate to promote leguminous growth.
5. Arylsulphatase and Chitinase activities were the most sensitive enzymes to SCs cultivation

Changes of soil biochemical properties as affected by subsidiary crop cultivation in four European pedo-climatic zones

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Abstract

Conservation agriculture represents an approach to managing agro-ecosystems aiming at preserving and enhancing the resource base and the environment. Subsidiary crops (SCs) are important components of conservation agriculture since they provide agro-ecological services, such as maintaining the soil resource. However, the importance of SC species and environment for the effect on soil microbial community are not well known. The overall objective of this study was to assess the effect of various subsidiary crops on soil microbial biomass and activity at four sites across Europe. The experiments were conducted during 2014 and 2015 at sites in the Nemoral (Sweden SLU), Oceanic (United Kingdom ORC), Continental (Switzerland AGS) and Mediterranean north (Italy UNI) pedo-climatic zones. The aims were to determine: (i) the effect of SC growth on soil microbial biomass and its activity, ii) the overall site-specific effect of the SCs growth of on soil biochemical properties. The SCs consisted of leguminous or brassicaceous species sown after harvest of wheat, or clover species under-sown in wheat. Microbial carbon and nitrogen quotients increased under SCs at the majority of sites indicating carbon and nitrogen immobilization process which may result in a positive trend of soil organic matter over time. Effects of SCs were similar in the pedo-climatic zones where temperatures are never below 0 °C (ORC and UNI). Moreover, arylsulphatase was the most sensitive enzyme to legumes in the Mediterranean north. In contrast, chitinase activity was enhanced by SCs in the Oceanic and Nemoral pedo-climatic zones. The high precipitation level and the low average temperature, typical of Continental and Nemoral zones, may represent limiting factors for soil enzyme activity under all selected SCs. Among the four pedo-climatic zones, the Mediterranean north represented the most suitable environment to promote SC growth and soil coverage. This study showed that SC cultivation affects soil quality enhancing biochemical activity; however this effect may be modulated by the different pedo-climatic conditions.

Keywords: subsidiary crop; microbial biomass; specific enzyme activity.

Introduction

Subsidiary crops (SCs) are grown primarily for their agro-ecological services. The adoption of SCs, in combination with minimum soil tillage practices and within a well-planned crop rotation, is the main pillar of conservation agriculture (Creamer and Dabney, 2002; Pittelkow et al., 2014). Subsidiary crops protect the soil from erosion, in particular during the fallow period between the two main cash crops, and they provide a continuum of root systems in soil, promoting soil microbial biomass and its activity through rhizo-deposition that provide uniform supply of organic C, as an energy source for microorganisms (Kumar et al., 2006; Paterson, 2013). The root system of SCs may increase soil microbial abundance and activity by enhancing the stabilization of soil macro-aggregates (Gyssels et al., 2005), which are 'hot spots' for soil microorganisms (Nannipieri et al., 2003; Sexstone et al., 1985). Some SCs with tap roots can penetrate deeply and help break up hard pans and bring nutrients up from deep layers while other SCs with fibrous roots, especially grasses, will increase soil carbon through their extensive root systems (Sarrantonio, 2012). Before cover crop suppression the presence of plant roots has a large impact on soil microbial communities and root exudates supply energy to soil microbes more efficiently than decomposing roots and crop residues (Calderon et al., 2016). In addition to their below ground effect, SCs can produce a large amount of above ground biomass. They promote nutrient cycling, and thus soil fertility, particularly when they are incorporated into the soil as green manure (Mancinelli et al., 2013; Fageria et al., 2005) or when they are mowed and left on the soil surface as organic dead mulch (Hartwig and Ammon, 2002).

Subsidiary crops can be non-leguminous species such as grasses (*Poaceae)* including cereals grown for that purpose, crucifers (*Brassicaceae*), other flowering plants, or legumes (*Fabaceae*). Leguminous species are widely used as SCs because of their ability to fix atmospheric nitrogen in symbiosis with Rhizobia. A large part of the N from legumes tends to be released soon after their suppression, as the residue decomposition process is generally

rapid mainly due to their low C/N ratio (Radicetti et al., 2016). Moreover, legumes have a greater positive effect on soil microbial biomass than other species due to a higher root exudation rate (Chen et al., 2008).

The impacts of SCs on soil nutrient biogeochemical cycling are usually documented in relation to the soil organic carbon pool variation (Mukumbareza et al., 2016) , which drives soil microbial activity, inducing a priming effect of native soil organic matter (SOM) (Insam and Domsch, 1988; Blagodatskaya and Kuzyakov, 2008; Murphy et al., 2011). Changes in agronomic practices may cause long-term changes of the total soil organic carbon content (Poeplau and Don, 2015). In the short-term, differences in soil C and N labile pools and soil enzyme activities can be used as indicators of biological activity and they are widely used to detect soil responses to agricultural management practices (Ramos et al., 2010; Zhou et al., 2012). The greater soil microbial biomass and activity occurring after SC suppression contribute to bio-geochemical nutrient cycling (Chavarria et al., 2016; Mbuthia et al., 2015). In this context, soil biochemical properties as related to soil microbial activity are often used as indicators of ecological changes and can be used to evaluate mineralization process dynamics based on substrate availability and seasonal fluctuations (Mancinelli et al., 2013; Marinari et al., 2015).

The benefits to the agro-ecosystem provided by SCs strongly depend on pedo-climatic conditions (Mondal et al., 2015), land use intensity (Wittwer et al., 2017) and SC type (Poffenbarger et al., 2015). These factors in turn affect crop productivity, the decomposition rates of SOM, and the abundance of substrates that can be directly used by the soil microbes (Davidson and Janssens, 2006; Marinari et al., 2015). Depending on SC species and pedo-climatic conditions SCs are likely to influence the biochemical properties differently. The effect after SC suppression on soil properties (i.e. the joint effects of the incorporation of the above ground biomass and roots after killing have been widely investigated (Poeplau

and Don, 2015). Conversely, there is only little knowledge about the effect of SC growth on soil nutrient availability and microbial biomass and activity.

The overall objective of this study was to fill this gap by assessing the effects of various standing SCs on soil carbon and nitrogen labile pools, and microbial activity. It was hypothesized that soil biochemical properties are influenced by SC species and pedo-climatic conditions. Therefore, coordinated field experiments were conducted at four sites located in different pedo-climatic zones across Europe. The effects were studied at the end of the cropping cycle of the SCs before soil tillage. In particular, the aims of the study were: (i) to assess the effect of SC root system before suppression on soil microbial biomass and its activity, in different pedo-climatic conditions (ii) to assess the site-specific effect of the annual meteorological conditions during the growth of the various SCs on soil biochemical properties.

2. Materials and methods.

2.1. Experimental setup and vegetation assessment

Field experiments were carried out in 2013/2014 and 2014/2015 (hereafter called cycle I and cycle II, respectively) at four sites (Figure 1). These sites represent a broad range of agro-environmental zones (Jongman et al., 2006): Nemoral (Swedish University of Agricultural Sciences, hereafter called SLU), Atlantic Central (Suffolk, United Kingdom - Organic Research Centre, ORC), Continental (Tänikon, Switzerland - Agroscope (AGS) and Mediterranean north (Viterbo, Italy - University of Tuscia, UNI). The pedo-climatic conditions at the four sites were quite different, they are summarized in table 1 and figure 1. As for climate description the aridity index (AI) was reported in addition to annual average of rainfall and temperature. The AI was calculated on a monthly basis as:

125 $$\mathbf{AI} = \frac{\mathbf{P}i}{\mathbf{T}i + \mathbf{10}}$$

126 where AI = aridity index; Pi = monthly precipitation amount; Ti = monthly mean air
127 temperature (Mancinelli et al., 2013). According to this index the wettest site, such as AGS,
128 shows the highest value in both crop cycles; while the driest sites were ORC in the cycle I and
129 UNI in cycle II. Finally, SLU showed the highest content of organic carbon (Corg) and an
130 acid pH. On the contrary UNI showed the lowest Corg content while ORC soil was the most
131 alkaline.

132 All sites followed a common design starting with wheat cultivated in the first year. Wheat was
133 sown either alone or intercropped with a leguminous species [subclover (*Trifolium*
134 *subterraneum* L.) at UNI and AGS; white clover (*Trifolium repens* L.) at SLU and yellow
135 trefoil (*Medicago lupulina* L.) at ORC] (Table 2). The leguminous species were chosen for
136 their abilities, either to self re-seed (subclover) or to re-grow (white clover and yellow trefoil),
137 in order to act as cover crop after wheat harvest. The pure wheat was followed either by a SC
138 sown immediately after harvest of wheat or bare (weedy) soil. The SCs sown after the wheat
139 were either the legume hairy vetch (*Vicia villosa* L. at SLU, AGS and UNI) or *Brassica* based
140 (Oilseed radish, *Raphanus sativus* L., at SLU and AGS and a *Brassica* mixture at ORC).
141 Moreover, a mixture of brassica and yellow trefoil was adopted as cover crop at ORC (Table
142 2). At each site, a soil without SCs was adopted as a control. In all experiments, the treatments
143 were replicated four times according to a randomized complete block design. In both crop
144 cycles, the percentage ground coverage of SC species was visually assessed at the end of SC
145 crop cycle. The soil coverage by the SCs differed among the four experimental sites. At ORC,
146 the brassica was partially killed due to frost over the winter. Similarly, at SLU the oilseed
147 radish and the hairy vetch were frost killed during winter. However, the hairy vetch recovered
148 to produce some biomass before being terminated (Figure 2).

149

150 *2.2. Soil chemical and biochemical properties*

151 Soil samples were collected at 0-30 cm depth before the establishment of the experimental
152 set-up in order to define the initial soil properties at each site. The second sampling was done
153 at the end of SC cultivation period, before the preparation of seed or planting bed for the
154 following main cash crop, in the spring of 2014 and 2015 for crop cycle I and II, respectively.
155 Three soil cores per plot were taken by means of an auger, air-dried before sieving (2 mm)
156 and preserved at room temperature for no more than four months. Soil moisture content was
157 adjusted to 60 % of the water holding capacity, and soil samples were then left to equilibrate
158 in the dark at room temperature for 3 days prior to measuring extractable C and N content,
159 microbial biomass and enzyme activities. Total organic carbon (Corg) and nitrogen (TN)
160 contents were determined using the dry combustion method with Thermo Soil NC—Flash
161 EA1112 elemental analyser (Tiessen & Moir, 1993). Each sample was pre-treated with a 10%
162 HCl solution to eliminate carbonates. In order to avoid differences induced by different soil
163 organic matter background between the sites, the soil labile pool and microbial activity were
164 expressed per unit of soil organic carbon. Microbial biomass carbon (Cmic) and nitrogen
165 (Nmic) were determined with the fumigation–extraction method (Vance et al., 1987), using
166 the (Shimadzu) TOC-V CSN and TNM-1 analyser. Microbial C (Cmic:Corg) and N index
167 (Nmic:TN) were calculated as a percentage of the total organic C and N. Additionally, the
168 extractable carbon (Extr C) and nitrogen (Extr N) were determined with the same equipment
169 on non-fumigated samples and expressed as a percentage of total organic C and N,
170 respectively.

171 The following hydrolytic enzymes, involved in soil biogeochemical cycles of the four main
172 elements (C, N, P and S), were analysed: for carbon β-glucosidase (EC 3.2.1.21), α-
173 glucosidase (EC 3.2.1.20), xylosidase (EC 3.2.2.27) and cellobiohydrolase (EC 3.2.1.91); for
174 nitrogen chitinase (EC 3.2.1.30), for phosphorus acid-phosphatase (EC 3.1.3.2); for sulphur
175 arylsulphatase (EC 3.1.6.1). Finally, the butyrate esterase (EC 3.1.1.1) was analysed as a

proxy of intracellular activity (Wittman et al., 2004). The enzyme activities were determined with the microplate assay (Marx et al., 2001) using fluorogenic substrates (4-MUF-β-D-cellobioside, 4-MUF-N-acetyl-β-glucosaminide, 4-MUF-β-D-glucoside, 4-MUF-α-D-glucoside, 4-MUF-phosphate, 4-MUF-sulphate, 4-MUF-7-β-D-xyloside and 4-MUF-butyrate as substrates). Fluorescence (excitation 360 nm, emission 450 nm) was measured with an automatic fluorimetric plate-reader (Fluoroskan Ascent) and readings were taken after 0, 30, 60, 120 and 180 min of incubation at 30 °C (Marinari et al., 2013). The enzyme activities were expressed per unit of soil organic carbon (specific enzyme activities) (Trasar-Cepeda et al., 2008) in order to compare the sites that presented different organic carbon contents. Moreover, the enzyme activities involved in the C cycle were expressed as Synthetic Enzyme Index (SEI C), which was calculated as the sum of 4 enzyme activities (β-glucosidase, α-glucosidase, xylosidase, cellobiohydrolase), releasing the same reaction product in the microplate fluorometric assay such as 4-methylumbelliferone (MUF). Finally, the soil microbial functional diversity was calculated using the Shannon Diversity Index calculated as $H' = -\sum pi \ln pi$ (Bending et al., 2002) where pi is the ratio of the activity of one enzyme to the sum of activities of all enzymes.

2.3 Data processing and statistical analysis

Analysis of variance (ANOVA) of chemical and biochemical soil properties data was performed separately for each crop cycle (I and II) and site. Fisher's protected least significant differences (LSD) at the 0.05 probability level ($P < 0.05$) were used for comparing the subsidiary crop treatments (leguminous CC, crucifers CC, living mulch and control). Normality of the data was checked using Kolmogorov-Smirnov test. The soil chemical and biochemical properties obtained in each experimental site were analysed using PCA in order to verify the effectiveness of the grouping variables to discriminate soil properties in the four

pedo-climatic zones. This analysis was applied separately for each SC treatment. The statistical analyses were performed using the JMP 9.0 statistical software package (SAS Institute, Cary, NC).

3. Results

The PCA score plot showed that the soil properties at the four sites were generally separated regardless of SCs (Figure 3). However, the clover treatments at ORC and UNI grouped together (Figure 3c). Based on the loading factor (Table 3) the main soil properties discriminating between the pedo-climatic zones were specific enzyme activities and the pool of labile nutrients, such as extractable and microbial carbon and nitrogen as percentage of total organic carbon. In contrast, the C:N ratio of both soil and microbial biomass together with the microbial basal respiration contributed little to the groups separation. The highest loading values on PC1 for each SC treatments and control soil were the specific enzyme activities (SEIC, Chit, Pho, Aryl). Moreover, the microbial carbon and nitrogen quotients Cmic:Corg and Nmic/TN, respectively can be included as loading factor for PC1 in case of clover living mulch and leguminous cover crops. Conversely, extractable C and N showed always high loading factors of PC2 for all treatments with positive coefficient in SC treatment and negative for control soil (Table 3).

Based on the site and cycle specific ANOVA, the soil microbial biomass and its activity together with the extractable C and N pools (Extr C and Extr N) were the most sensitive soil indicators showing significant differences among SC treatments at each site (Table 4). Conversely, as expected, soil total organic C and total N did not change due to SC treatments. Moreover, the Extr C and Extr N, expressed per gram of soil, were affected by SC treatment, while they were less sensitive to SC treatment when they were expressed per unit of organic carbon and total nitrogen, respectively (Table 4). The only significant differences for Extr

N/TN were observed in samples from cycle I at AGS and cycle II at UNI, and for Extr C/Corg in cycle II at AGS. In particular, the variation of the extractable N pool as percentage to the total N was caused by leguminous SCs, hairy vetch and subclover. At UNI, Extr N/TN under vetch was 4.0 % and with subclover 3.5 % vs. 3.0 % of control, while in the first cycle of AGS, the Extr N/TN under vetch was 5.5% and 4.9 % under subclover vs. 4.1% in the control. In the cycle II at AGS a significant increase of Extr C/Corg was also observed after brassica and subclover, 1.4% and 1.3%, respectively compared to 0.7% in the control soil. The percentage of extractable fractions to the total C and N content changed across the pedo-climatic zones reaching the lowest values under Nemoral climatic conditions (SLU), while the highest values were observed at the Mediterranean north site (UNI) (Fig. 4). Conversely, the SC treatment significantly increased the microbial carbon quotient (Cmic:Corg) at the Oceanic and Mediterranean north sites (ORC and UNI, respectively) in both crop cycles. In the two years 2014 and 2015, similar SC effects were found on microbial nitrogen quotient (Nmic:TN) in the Continental and Nemoral sites (AGS and SLU, respectively). The Cmic:Corg was sensitive to brassica at ORC and to Vetch and subclover at UNI with a higher value with respect to the control soil (Fig. 4a). Moreover, in the crop cycle I at the AGS site, vetch resulted in the highest microbial carbon quotient. The microbial nitrogen quotient (Nmic:TN) at AGS increased in all SC treatments compared to the control soil, while at SLU the brassica resulted in a high percentage at both crop cycles (Fig. 4b).

Several significant differences occurred at the Oceanic and Mediterranean north sites (ORC and UNI) (Table 4). In particular, enzymes involved in C (SEIC), S (arylsulphatase) and N (chitinase) cycles were affected by SC treatments (Table 4). Repeated effects across crop cycles occurred only for chitinase activity in the northern sites (ORC and SLU) and for arylsulphatase activity at the southern site (UNI) (Table 4). Other significant differences in enzyme activity occurred between SC treatments with respect to chitinase an enzyme involved in the carbon and nitrogen bio-geo-chemical cycle. The activity of this enzyme was

252 particularly enhanced in soil under vetch at the Nemoral site (SLU) and in soil under vetch
253 and subclover at the Mediterranean north site (UNI) (Fig. 5c). Moreover, the specific activity
254 of acid phosphomonoesterase was similar in all SC treatments at UNI and AGS while at ORC
255 the effect of brassica was significant only at the cycle I and at SLU the effect of SC treatment
256 was evident only at the cycle II. At UNI the specific activity of arylsulphatase was highly
257 positively affected by the leguminous SCs (Fig. 5d). As an overall observation, ORC and UNI
258 had the highest microbial biomass (C and N) (Fig. 5).

259 Trends for extractable C and N, expressed as percentage to the respective total amount of C
260 and N, differed among sites (Fig. 6 a and b). While Extr C/Corg tended to be similar among
261 sites (Fig. 6a), Extr N/TN was highest in the Mediterranean north and lowest in the Nemoral
262 pedo-climatic zone (Fig.6b).

263 The specific soil enzyme activities mainly varied between cycles I and II as well as among the
264 four pedo-climatic zones as shown by the synthetic enzyme index (Fig. 7a). Moreover, the
265 microbial functional diversity expressed by the Shannon index (H') was slightly higher at
266 AGS compared to the other sites (Fig. 7b). The ANOVA revealed that for H' differences
267 along the two cropping cycles were significant only at the Mediterranean north site UNI
268 (Table 4) where SC treatments enhanced the microbial biomass functional diversity (Vetch
269 2.18 and subclover 1.98 vs. Control soil 1.90).

270

271 **4. Discussion**

272 In this study, the SC effect was observed at the end of SC cultivation period, before the
273 preparation of planting bed of the following main cash crop. This allowed evaluating the
274 combined effects of SC cultivation with pedo-climatic conditions on soil labile C and N pools
275 and microbial activity over a two-year period. This is in contrast to the majority of studies that
276 investigate the effects of SCs after their suppression and during the following main cash crop

277 cultivation when the SC residues were either left on the soil surface, as dead mulch, or
278 incorporated into the soil as green manure. This does not allow to separate effects of the
279 mineralization process of the residues from the plant effects themselves (Radicetti et al., 2016;
280 Marinari et al., 2015; Dinesh et al., 2001; Hu et al. 1997). SC suppression means a rapid and
281 substantial input of organic matter usually during periods with climatic conditions conducive
282 to microbial degradation processes, to support rapid microbial processes of incorporation of
283 organic matter. The usually slower and more variable processes of rhizodeposition and other
284 microbial processes during SC growth, as influenced by pedo-climatic conditions, become,
285 thus, indiscernible. Therefore, this work provides insight on soil biochemical changes induced
286 by the species specific effects that SCs produce during their growth.

287

288 *4.1 Effect of pedo-climatic zones on soil biochemical properties across Europe*

289 As the two cycles were conducted in different fields at the different sites, pedo-climatic and
290 weather conditions cannot be fully separated. The interaction of pedo-climatic zones and SC
291 species resulted in soil changes in terms of nutrient labile pool and biochemical activity and it
292 was considered SC specific when the effect occurred in both crop cycles (I and II) although
293 microbial population size and activity was shown to be very sensitive to seasonal fluctuation
294 at UNI (Marinari et al., 2015). Therefore, the repeatability of the effects of SC growth on soil
295 microbial biomass under different meteorological conditions suggests a dominant effect of SC
296 on soil biochemical properties.

297 In the Mediterranean north and Oceanic pedo-climatic zones, where the aridity index (AI) was
298 lower and temperatures were seldom below 0 ºC, the microbial indices (Cmic:Corg;
299 Nmic:TN) and the synthetic enzymatic index (SEI) were higher than in the Continental and
300 Nemoral zones. This may be because soil microorganisms rapidly metabolize labile substrates
301 when temperature are mild (Davidson and Janssens, 2006; Marinari et al., 2015). Increased

302 precipitation and temperature enhance the soil labile pool of C and N, which are easily
303 decomposable by soil microorganisms and an important source of nutrients for the agro-
304 ecosystems (Song et al., 2012). In this study, the effect of pedo-climatic conditions was
305 particularly evident for Extr N/TN being the highest in the Mediterranean north zone.
306 Moreover, in the Oceanic and Mediterranean north zones, effects of clover were almost
307 equivalent making soils of the two pedo-climatic zones more similar compared to the other
308 SCs and control treatments. This effect might be due to the fact that similar climate conditions
309 allowed clover to grow more vigorously on the different soils of those areas, where
310 temperature was never below 0 °C and a low AI was registered. Conversely, spring-sown
311 clover never died at SLU, but its growth was prevented by low temperatures. Soil freezing has
312 a direct influence on the soil water availability, causing a slower diffusion of C based
313 substrates and enzymes within the soil matrix (Jefferies et al., 2010). Moreover, very cool or
314 frozen soil either slows down microbial metabolism or may kill microorganisms due to cell
315 starvation or rupture (Jefferies et al., 2010). Finally, in the Continental pedo-climatic zone
316 (AGS) the soil specific enzyme activity was generally the lowest among all sites, probably
317 due to the highest precipitation level observed in this site during SCs cultivation. Soil water
318 *status* is an important aspect affecting microbial community activity (Pan et al., 2016) and
319 structure since they are partly controlled by soil oxygen availability, which is in turn
320 controlled by soil moisture (Drenovsky et al. 2004; Schimel et al. 2007).

321

322 *4.2 Effect of SC cultivation on soil biochemical properties*

323 In general, positive effects on soil biochemical characteristics associated to the
324 biogeochemical cycles of nutrients, were observed after vetches and brassica-trefoil mixtures
325 cultivation than after brassica. Although in this study no data on root exudates were collected,
326 it is likely that the different effects of various SC species on microbial biomass pool and its

activity, could be due to the rhizosphere effect and root exudates that led to changes in the composition, size and activity of the soil microflora (Chavarria et al., 2016). In the Mediterranean north environment (UNI), both leguminous SCs (vetch and subclover) resulted in an increase of microbial biomass and their functional diversity (H') in both crop cycles I and II. Enhancing plant diversity increases soil microbial diversity, therefore populations of beneficial microbes such as disease-suppressive bacteria can be increased by increasing plant functional group richness including SCs in the crop rotation (Vukicevich et al., 2016). Similarly, in the Continental environment (AGS) an increase of microbial nitrogen quotient (Nmic:TN) was observed after vetch.

The significant positive effect on the soil microbial pool by leguminous SCs, especially vetch, observed in the Mediterranean north and Continental pedo-climatic zones (UNI and AGS), could probably be explained by the vigorous growth of the SCs, as indicated by the high soil coverage, and the associated well-developed root system. Moreover, Leguminous species have a great diversity of root exudates (Sugiyama and Yazaki, 2012) attracting a larger amount of microorganisms compared with other SC families. This might explain the higher microbial quotient and activity under vetch and subclover.

The site-specific effect of SC due to fields or annual meteorological conditions was evident when a different response of labile nutrient pools and enzyme activities was found between the crop cycles I and II. Repeated positive effects (both in cycle I and II) on arylsulphatase activity were found in the Mediterranean north environment. Conversely, in the Nemoral and Oceanic pedo-climatic conditions (SLU and ORC sites), where SCs coverage was reduced with respect to the other sites, a repeated positive effect (both in cycle I and II) of SCs was registered for chitinase activity. The increase of arylsuphatase activity observed in soil under vetch at UNI may suggest a high demand of S by this cover crop in the environment where its growth is particularly enhanced. S in soil might promote root nodule growth and thus the growth of legumes (Latef and Ahmad, 2015). Low concentrations of SO_4 in soils could

353 stimulate soil microbes to release arylsuphatase as extracellular enzyme (Saviozzi et al., 2006;
354 Wilhelm, 2009).

355 In the Nemoral and Oceanic climatic zones most of the specific enzyme activities were
356 sensitive to annual conditions, with the exception of chitinase activity. It is known that soil
357 nitrogen availability usually increases under legumes SC (Radicetti et al., 2016). This in turn
358 may act as a negative feedback on soil chitinase activity (Olander and Vitousek, 2000) while
359 promoting activity of other soil enzyme activity, such as phosphatase (Olde Venterink, 2011).
360 Moreover, the increase of chitinase activity can be attributed to the constant presence of
361 fungal populations in soils (Vepsäläinen, 2012) that could be particularly high in soil under
362 SC's in Nemoral and Oceanic pedo-climatic zones. Conversely, in Mediterranean pedo-
363 climatic zone, mild temperatures may promote bacterial community growth characterized by a
364 faster metabolism than other microbial groups (Pietikainen et al., 2005). Moreover, in the
365 same pedo-climatic zones, brassica SC had a positive effect on soil microbial C and N pools.
366 Previous studies reported that the size of microbial C and N pools are not only affected by
367 climate and crop but also by the growth stage of the brassicas with a higher biomass around
368 stem elongation (Sabahi et al., 2010). Therefore, even if the SCs at ORC and SLU sites did
369 not achieve complete soil coverage, the root system established at the end of the cover crop
370 cycle may have promoted soil microbial biomass and activity with particular effects on
371 chitinase activity. Finally, the increase of Cmic:Corg observed at all sites suggests a carbon
372 immobilization process within the microbial biomass. This may lead to a positive future trend
373 on soil C storage due to SCs. Cmic:Corg is an early and sensitive predictor of changes
374 occurring in soil organic matter making it a useful parameter for short-term studies that do not
375 allow the assessment of TOC changes (Marinari et al., 2006; Lagomarsino et al., 2009). The
376 lack of significant effect of SCs on the carbon labile fraction (Extr C/Corg) supports the
377 efficacy of this indicator suggesting that no losses as soluble carbon forms occurred.

378

5. Conclusions

In conclusion, SC cultivation positively affected soil microbial biomass and activity. Even though microbial population size and activity are sensitive to seasonal fluctuations, a dominant effect of SCs, independent of annual meteorological conditions, was found within climatic zones. Depending on climatic zone, either arylsulphatase (Mediterranean north zone) or chitinase enzymes (Oceanic, Nemoral zone) were enhanced by SCs species. The microbial C and N immobilization occurring during SC cultivation represents an early predictor of positive effects on soil organic matter in the long term.

Among the four pedo-climatic zones, the growth of legumes as living mulch enhances soil specific enzyme activity producing similar effects in pedo-climatic zones where the mean temperatures do not fall below 0 °C (Mediterranean north and Oceanic, respectively). Moreover, the Mediterranean north is the most suitable to promote growth and coverage of leguminous SCs, enhancing soil microbial activity and functional diversity. As for Continental and Nemoral zones, the high precipitation level and the low average temperature, respectively represent limiting factors for soil enzyme activity under all selected SCs.

Subsidiary crops utilization is confirmed to be an effective agricultural practice enhancing soil biochemical properties even before their suppression. However, it is important to evaluate their potential beneficial effect in relation to the specific pedo-climatic area where the positive effects may have different extent.

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Bibliography

Bending, G.D., Turner, M.K., Jones, J.E., 2002. Interactions between crop residue and soil organic matter quality and the functional diversity of soil microbial communities. Soil Biol. Biochem. 34, 1073–1082. doi:http://dx.doi.org/10.1016/S0038-0717(02)00040-8

Blagodatskaya, E., Kuzyakov, Y. 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: Critical review. Biol Fertil Soils 45(2), 115-131

Calderon, F.J., Nielsen, D., Acosta-Martinez, V., Vigil, M.F., Lyon, D., 2016. Cover crop and irrigation effects on soil microbial communities and enzymes in semiarid agroecosystems of the central great plains of north america. Pedosphere 26, 192–205. doi:10.1016/S1002-0160(15)60034-0

Chavarria, D.N., Verdenelli, R.A., Serri, D.L., Restovich, S.B., Andriulo, A.E., Meriles, J.M., Vargas-Gil, S., 2016. Effect of cover crops on microbial community structure and related enzyme activities and macronutrient availability. Eur. J. Soil Biol. 76, 74–82. doi:10.1016/j.ejsobi.2016.07.002

Chen, M., Chen, B., Maeschener, P., 2008. Plant growth and soil microbial community structure of legumes and grasses grown in monoculture or mixture. J. Environ. Sci. 20, 1231–1237. doi:10.1016/S1001-0742(08)62214-7

Creamer, N.G., Dabney, S.M., 2002. Killing cover crops mechanically: review of recent literature and assessment of new research results. Am. J. Altern. Agr. 17, 32–40. doi:10.1079/AJAA20014

426 Davidson, E.A., Janssens, I.A., 2006. Temperature sensitivity of soil carbon decomposition
427 and feedbacks to climate change. Nature 440, 165–173.

428 Dinesh, R., Suryanarayana, M.A., Nair, A.K., Ghoshal Chaudhuri, S., 2001. Leguminous
429 cover crop effects on nitrogen mineralization rates and kinetics in soils. Journal of Agronomy
430 and Crop Science 187(3), 161-166.

431 Drenovsky, R.E., Vo, D., Graham, K.J., Scow, K.M., 2004. Soil water content and organic
432 carbon availability are major determinants of soil microbial community composition, Microb.
433 Ecol., 48, 424-430. DOI: 10.1007/s00248-003-1063-2.

434 Fageria, N.K., Baligar, V.C., Bailey, B.A., 2005. Role of cover crops in improving soil and
435 row crop productivity. Comm. Soil Sci. Plant Anal. 36, 2733–2757.
436 doi:10.1080/00103620500303939

437 Gyssels, G., Poesen, J., Bochet, E., Li, Y., 2005. Impact of plant roots on the resistance of
438 soils to erosion by water: a review. Prog. Phys. Geog.29, 189–217.
439 doi:10.1191/0309133305pp443ra

440 Hartwig, N.L., Ammon, H.U., 2002. Cover crops and living mulches. Weed Sci. 50, 688–699.

441 Iizumi, T., Ramankutty, N., 2015. How do weather and climate influence cropping area and
442 intensity? Global Food Sec. 4, 46–50. doi:10.1016/j.gfs.2014.11.003

443 Hu, S., Grunwald, N.J., Van Bruggen, A.H.C., (...), Shennan, C., Demment, M.W., 1997.
444 Short-term effects of cover crop incorporation on soil carbon pools and nitrogen availability.
445 Soil Science Society of America Journal 61(3), 901-911.

446 Insam, H., Domsch, K.H., 1988. Relationship between soil organic carbon and microbial
447 biomass on chronosequences of reclamation sites. Microb. Ecol. 15, 177–88.
448 doi:10.1007/BF02011711

449 Jefferies, R.L., Walker, N.A., Edwards, K.A., Dainty, J., 2010. Is the decline of soil microbial
450 biomass in late winter coupled to changes in the physical state of cold soils? Soil Biol.
451 Biochem. 42, 129–135. doi:10.1016/j.soilbio.2009.10.008

452 Jongman, R.H.G., Bunce, R.G.H., Metzger, M.J., Mucher, C.A., Howard, D.C., Mateus, V.L.,
453 2006. Objectives and applications of a statistical environmental stratification of Europe.
454 Landscape Ecol. 21, 409–419. doi:10.1007/s10980-005-6428-0

455 Kumar, R., Pandey, S., Pandey, A., 2006. Plant roots and carbon sequestration. Curr. Sci. 91,
456 885–890.

457 Lagomarsino, A., Moscatelli, M.C., Di Tizio, A., Mancinelli, R., Grego, S**.,** Marinari, S.,
458 2009. Soil biochemical indicators as a tool to assess the short-term impact of agricultural
459 management on changes in organic C in a Mediterranean environment. Ecol. Ind., 9(3), 518-
460 527. doi:10.1016/j.ecolind.2008.07.003

461 Latef, A.A.H.A., Ahmad, P., 2015. Legumes and breeding under abiotic stress in: Azooz, M.
462 M., Ahmad, P. (Eds): Legumes under Environmental Stress. John Wiley & Sons, Ltd,
463 Chichester, UK pp. 60–61. doi:10.1002/9781118917091.ch1

464 Mancinelli, R., Marinari, S., Di Felice, V., Savin, M.C., Campiglia, E., 2013. Soil property,
465 CO_2 emission and aridity index as agroecological indicators to assess the mineralization of
466 cover crop green manure in a Mediterranean environment. Ecol. Ind. 34, 31–40.
467 doi:10.1016/j.ecolind.2013.04.011

468 Marinari, S., Mancinelli, R., Campiglia, E., Grego, S., 2006. Chemical and biological
469 indicators of soil quality in organic and conventional farming systems in Central Italy. Ecol.
470 Ind., 6, 701-711. doi.org/10.1016/j.ecolind.2005.08.029

471 Marinari, S., Bonifacio, E., Moscatelli, M.C., Falsone, G., Antisari, L.V., Vianello, G., 2013.
472 Soil development and microbial functional diversity: proposal for a methodological approach.

473 Geoderma 192, 437–445. doi:10.1016/j.geoderma.2012.08.023

474 Marinari, S., Mancinelli, R., Brunetti, P., Campiglia, E., 2015. Soil quality, microbial
475 functions and tomato yield under cover crop mulching in the Mediterranean environment. Soil
476 Till. Res. 145, 20–28. doi:http://dx.doi.org/10.1016/j.still.2014.08.002

477 Marx, M.C., Wood, M., Jarvis, S.C., 2001. A microplate fluorimetric assay for the study of
478 enzyme diversity in soils. Soil Biol. Biochem. 33, 1633–1640. doi:doi: 10.1016/s0038-
479 0717(01)00079-7

480 Mbuthia, L.W., Acosta-Martínez, V., DeBruyn, J., Schaeffer, S., Tyler, D., Odoi, E.,
481 Mpheshea, M., Walker, F., Eash, N., 2015. Long term tillage, cover crop, and fertilization
482 effects on microbial community structure, activity: implications for soil quality. Soil
483 Biol.Biochem. 89, 24–34. doi:10.1016/j.soilbio.2015.06.016

484 Mondal, P., Jain, M., DeFries, R.S., Galford, G.L., Small, C., 2015. Sensitivity of crop cover
485 to climate variability: Insights from two Indian agro-ecoregions. J Environ Manage 148, 21-
486 30

487 Mukumbareza, C., Muchaonyerwa, P., Chiduza, C., 2016. Bicultures of oat (Avena sativa L.)
488 and grazing vetch (Vicia dasycarpa L.) cover crops increase contents of carbon pools and
489 activities of selected enzymes in a loam soil under warm temperate conditions. Soil Sci. Plant
490 Nutr. 62, 447–455. doi:10.1080/00380768.2016.1206833

491 Murphy, D. V., Cookson, W.R., Braimbridge, M., Marschner, P., Jones, D.L., Stockdale,
492 E.A., Abbott, L.K., 2011. Relationships between soil organic matter and the soil microbial
493 biomass (size, functional diversity, and community structure) in crop and pasture systems in a
494 semi-arid environment. Soil Res. 49, 582–594. doi:10.1071/SR11203

495 Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G., Renella, G., 2003.
496 Microbial diversity and soil functions. Eur. J. Soil Sci. 54, 655–670. doi:10.1046/j.1351-

0754.2003.0556.x

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

Olde Venterink, H., 2011. Legumes have a higher root phosphatase activity than other forbs, particularly under low inorganic P and N supply. Plant Soil 347, 137–146. doi:10.1007/s11104-011-0834-7

Pan, F., Li, Y., Chapman, S. J. , Yao, H., 2016. Effect of rice straw application on microbial community and activity in paddy soil under different water status. Env. Sci. Pollut. R., 23, 6, 5941–5948. DOI: 10.1007/s11356-015-5832-5

Paterson, E., 2013. The Use of Stable Isotope Labeling and Compound-Specific Analysis of Microbial Phospholipid Fatty Acids to Quantify the Influences of Rhizodeposition on Microbial Community Structure and Function in: de Bruijn F.S. (Ed.), Molecular microbial ecology of the rhizosphere, John Wiley & Sons, vol. 1, pp. 141–149.

Pietikainen, J., Pettersson, M., Baath, E., 2005. Comparison of temperature effects on soil respiration and bacterial and fungal growth rates. FEMS Microbiol. Ecol. 52, 49–58. doi:10.1016/j.femsec.2004.10.002

Pittelkow, C.M., Liang, X., Linquist, B.A., van Groenigen, K.J., Lee, J., Lundy, M.E., van Gestel, N., Six, J., Venterea, R.T., van Kessel, C., 2015. Productivity limits and potentials of the principles of conservation agriculture. Nature 517, 365–368.

Poeplau, C., Don, A., 2015. Carbon sequestration in agricultural soils via cultivation of cover crops - A meta-analysis. Agric., Ecosyst. Environ. 200, 33–41. doi:10.1016/j.agee.2014.10.024

Poffenbarger, H.J., Mirsky, S.B., Weil, R.R., (...), Spargo, J.T., Cavigelli, M.A., 2015. Biomass and nitrogen content of hairy vetch-cereal rye cover crop mixtures as influenced by

521 species proportions. Agronomy Journal 107(6), 2069-2082.

522 Radicetti, E., Mancinelli, R., Moscetti, R., Campiglia, E., 2016. Management of winter cover
523 crop residues under different tillage conditions affects nitrogen utilization efficiency and yield
524 of eggplant (*Solanum melanogena L.)* in Mediterranean environment. Soil Till. Res. 155,
525 329–338. doi:10.1016/j.still.2015.09.004

526 Ramos, M.E., Benítez, E., García, P. a., Robles, A.B., Garcı, P.A., Ramos, E., Benı, E.,
527 Ramos, M.E., Benítez, E., García, P. a., Robles, A.B., 2010. Cover crops under different
528 managements vs. frequent tillage in almond orchards in semiarid conditions: effects on soil
529 quality. Appl. Soil Ecol. 44, 6–14. doi:10.1016/j.apsoil.2009.08.005

530 Sabahi, H., Veisi, H., Soufizadeh, S., Asilan, K.S., 2010. Effect of fertilization systems on soil
531 microbial biomass and mineral nitrogen during canola (Brassica napus L.) development
532 stages. Comm. Soil Sci. Plant Anal. 41, 1665–1673. doi:10.1080/00103624.2010.489132

533 Sarrantonio M., 2012. Building soil fertility and tilth with cover crops, in Clark A. (Ed.):
534 Managing cover crops profitably, 3rd edition, Sustainable Agriculture Research and
535 Education Program, Washington DC, pp 16-24

536 Saviozzi, A., Cardelli, R., N'kou, P., Levi-Minzi, R., Riffaldi, R., 2006. Soil biological
537 activity as influenced by green waste compost and cattle manure. Compost Sci. Util. 14, 54–
538 58. doi:10.1080/1065657X.2006.10702263

539 Schimel, J., Balser, T.C., Wallenstein, M., 2007. Microbial stress-response physiology and
540 its implications for ecosystem function, Ecology, 88, pp. 1386-1394. DOI: 10.1890/06-0219

541 Sexstone, A.J., Parkin, T.B., Tiedje, J.M., 1985. Temporal Response of Soil Denitrification
542 Rates to Rainfall and Irrigation1. Soil Sci. Soc. Am. J. 49, 99–103.
543 doi:10.2136/sssaj1985.03615995004900010020x

544 Song, B., Niu, S., Zhang, Z., Yang, H., Li, L., Wan, S., 2012. Light and heavy fractions of

545 soil organic matter in response to climate warming and increased precipitation in a temperate
546 steppe. PLOS ONE 7, e33217.

547 Sugiyama, A., Yazaki, K., 2012. Secretions and exudates in biological systems.In Vivanco, J.
548 M and Baluška, F (Eds.): Signaling and Communication in Plants, Springer Berlin
549 Heidelberg, vol. 12, pp 27–49. doi:10.1007/978-3-642-23047-9

550 Tiessen, H., and Moir, J.O. 1993. Total and organic carbon, in: Carter, M.R. (Ed.), Soil
551 sampling and Methods of Analysis. Lewis Publishers, pp. 187-200.

552 Trasar-Cepeda, C., Leirós, M.C., Gil-Sotres, F., 2008. Hydrolytic enzyme activities in
553 agricultural and forest soils. Some implications for their use as indicators of soil quality. Soil
554 Biol. Biochem. 40, 2146–2155. doi:10.1016/j.soilbio.2008.03.015

555 Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil
556 microbial biomass C. Soil Biol. Biochem. 19, 703–707. doi:http://dx.doi.org/10.1016/0038-
557 0717(87)90052-6

558 Vepsäläinen, M., 2012. Functional biodiversity in soils : Development and applicability of an
559 enzyme activity pattern measurement method. Monographs of the Nemoral Env. Res. No. 41.

560 Vukicevich, E., Lowery, T., Bowen, P., Úrbez-Torres, J.R., Hart, M., 2016. Cover crops to
561 increase soil microbial diversity and mitigate decline in perennial agriculture. A review.
562 Agron. Sustainable Dev. 36, 48. doi:10.1007/s13593-016-0385-7

563 Wilhelm Scherer, H., 2009. Sulfur in soils. J. Plant Nutr.Soil Sci. 172, 326–335.
564 doi:10.1002/jpln.200900037

565 Wittmann, C., Kahkonen, M.A., Ilvesniemi, H., Kurola, J., Salkinoja-Salonen, M.S., 2004.
566 Areal activities and stratification of hydrolytic enzymes involved in the biochemical cycles of
567 carbon, nitrogen, sulphur and phosphorus in podsolized boreal forest soils. Soil Biol.
568 Biochem. 36, 425–433.

569 Wittwer, R.A., Dorn, B., Jossi, W., van der Heijden, M.G.A., 2017. Cover crops support
570 ecological intensification of arable cropping systems. Scientific Reports 7:41911 | DOI
571 10.1038/srep41911

572 Zhou, X., Wu, H., Koetz, E., Xu, Z., Chen, C., 2012. Soil labile carbon and nitrogen pools and
573 microbial metabolic diversity under winter crops in an arid environment. Appl. Soil Ecol. 53,
574 49–55. doi:10.1016/j.apsoil.2011.11.002

575

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Table
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Table 1. Pedo-climatic description of the four European experimental sites.

		Climate description (*)			Soil properties							
Crop cycles	Sites	Rainfall (avg mm y^{-1})	Temperature (avg °C y^{-1})	AI	Clay (%)	Silt (%)	Sand (%)	Soil texture (USDA)	pH (H_2O) 1:2.5 w:v	Total carbonate	Corg	TN
I (2013-2014)	SLU	598	8.2	2.66	10	60	24	Silt loam	5.7	1.4	30.8	2.8
	ORC	628	10.8	1.19	58	20	22	Clay	7.5	4.6	20.7	2.0
	AGS	1111	9.5	3.74	19	35	46	Loam	7.1	1.0	20.1	2.2
	UNI	845	11.6	2.84	23	21	55	Sandy-Clay-loam	6.7	0.4	11.0	1.1
II (2014-2015)	SLU	526	7.3	1.18	20	63	14	Silt loam	6.1	3.0	28.6	2.2
	ORC	662	10.5	0.95	58	20	22	Clay	7.2	2.8	23.2	2.7
	AGS	1259	10.6	4.80	22	35	43	Loam	6.9	4.5	21.6	2.3
	UNI	614	11.2	0.86	15	22	63	Sandy-loam	6.7	1.0	12.1	1.1

*Averages of rainfall and temperature are calculated considering data record of 12 months before soil sampling date, AI= aridity index is calculated on a monthly basis (30 days before soil sampling)

Corg = total organic carbon, TN = total nitrogen.

Table 2. Description of experimental set-up of crop cycle I and II.

Crop cycle	Sites	Main Crop1	Subsidiary Crops	Soil sampling
I	SLU	Soft Wheat	Oilseed radish Hairy vetch White clover	05.05.2014
	ORC	Soft Wheat	Brassica mixture[1] Brassica+Yellow trefoil Yellow trefoil	03.04.2014
	AGS	Soft Wheat	Oilseed radish Hairy Vetch Subclover	19.05.2014
	UNI	Durum Wheat	Hairy Vetch Subclover	24.04.2014
II	SLU	Soft Wheat	Oilseed radish Hairy vetch White clover	05.05.2015
	ORC	Soft Wheat	Brassica mixture[1] Brassica+Yellow trefoil Yellow trefoil	10.04.2015
	AGS	Soft Wheat	Oilseed radish Hairy Vetch Subclover	02.06.2015
	UNI	Durum Wheat	Vetch Subclover	29.04.2015

[1]Forage rape, White mustard and Fodder radish

Table 3. Loading factor values of the PCA related to figure 3; leguminous spp. (A), brassicaceous sp (B), clovers (C) and control plots (D). C/N = carbon to nitrogen ratio, Cmic:Corg = microbial carbon to total organic carbon ratio, Nmic:TN = microbial nitrogen to total nitrogen ratio, Ext C/Corg = extractable carbon to total organic carbon ratio, Ext N/TN = extractable nitrogen to total nitrogen ratio, Cmic/Nmic = microbial carbon to microbial nitrogen ratio, SEIC/Corg = sum of C cycle enzymes to total organic carbon ratio (C cycle enzymes specific activity), Chit/Corg = chitinase activity to total organic carbon ratio, Pho/Corg = phosphatase activity to total organic carbon ratio, Aryl/Corg = arylsulphatase activity to total organic carbon ratio, H' = Shannon diversity index.

	A		**B**		**C**		**D**	
	PC 1	PC 2	PC 1	PC 2	PC 1	PC 2	PC 1	PC 2
C/N	0.7	-0.3	0.6	-0.6	0.3	-0.7	0.1	0.8
Cmic:Corg	0.5	0.6	-0.3	0.9	0.5	0.7	0.2	-0.4
Nmic/TN	0.3	0.8	0.4	0.8	0.8	0.4	0.9	-0.2
Ext C/Corg	0.0	0.8	-0.6	0.7	0.1	0.7	0.4	-0.7
Ext N/TN	0.2	0.8	-0.6	0.5	0.1	0.8	0.4	-0.7
Cmic/Nmic	0.7	0.0	-0.8	0.1	-0.3	0.2	-0.6	0.2
SEIC/Corg	0.8	0.0	0.7	0.6	0.9	0.0	0.9	0.2
Chit/Corg	0.8	-0.2	0.7	0.7	0.9	-0.1	0.8	0.3
Pho/Corg	0.8	-0.1	0.9	0.1	0.6	-0.7	0.6	0.7
Aryl/Corg	0.6	0.1	0.6	0.0	0.5	-0.2	0.1	0.5
H'	-0.6	0.5	-0.4	-0.6	-0.7	0.0	-0.6	-0.3

Table 4. Statistical analysis (ANOVA) of SCs effect on soil chemical and biochemical properties at the end of SCs crop cycle in the four European experimental sites (* p<0.05; ** p<0.01; *** p<0.001). For acronyms see table 3

	SLU		**ORC**		**AGS**		**UNI**	
	Cycle I	Cycle II	Cycle I	Cycle II	Cycle I	Cycle II	Cycle I	Cycle II
Corg								
TN								
Extr C				***		*		*
Extr N			*			***	***	***
Extr C/Corg						**		
Extr N/TN					**			**
Cmic		***	***	**	***		***	**
Cmic:Corg		**	***	*	**		***	**
Nmic	*	***	**	*	*	*		
Nmic/TN	*	***	**		*	***		*
Cmic:Nmic		***	**		*	*	*	
SEI/Corg			***					*
SEIC/Corg			***					
Chit/Corg	**	*	***	*				**
Pho/Corg		*	*					
Aryl/Corg			**				**	***
H’							*	*

Figure captions

Figure 1. Localization and weather conditions (monthly average of the daily temperatures and monthly total amount of rainfall) during the field experiments in 2013/2014 and 2014/2015 years of the four experimental sites.

Figure 2. Soil coverage of the SC treatments: brassica sp. (Br), brassica and yellow trefoil mixture (Br YT), vetch (V), subclover (Sub C), white clover (Wh C), and control (C) in the four pedo-climatic zones: SLU (Nemoral); ORC (Oceanic); AGS (Continental); and UNI (Mediterranean north), for crop cycles I (dark grey) and II (light grey). Bars represent the standard error (n=4).

Figure 3. Principal component analysis (PCA) of the four SC treatments: annual leguminous (a), brassicaceous crops (b) sown after harvest of wheat, clovers under-sown in wheat (c), and control soil (d) over both crop cycles. The markers representing the experimental sites were: circles-AGS (Continental); squares-ORC (Oceanic); triangles-SLU (Nemoral) and asterisk UNI (Mediterranean north). For acronyms see table 3.

Figure 4. (A) Microbial carbon (Cmic:Corg) and (B) nitrogen (Nmic/TN) quotients after SC treatments: brassica spp. (Br), brassica and yellow trefoil mixture (Br YT), vetch (Ve), subclover (Sub C), white clover (Wh C), and control (C), across the four pedo-climatic zones SLU (Nemoral), ORC (Oceanic), AGS (Continental), and UNI (Mediterranean north), for crop cycles I (dark grey) and II (light grey). Bars represent the standard error (n=4).

Figure 5. Soil enzyme activities involved in biogeochemical cycles and their functional diversity: (A) C-cycle enzyme activities (SEI C); (B) chitinase (Chit), (C) acid phosphomonoesterase (Pho), and (D) arylsulphatase (Aryl). The activities are expressed per unit of soil organic carbon. The CC treatments considered: brassica sp. (Br), brassica and yellow trefoil mixture (Br YT), vetch (V), subclover (Sub C), white clover (Wh C), and control (C) in the four pedo-climatic zones: Nemoral

(SLU), Oceanic (ORC), Continental (AGS) and Mediterranean north (UNI) at both crop cycles I (dark grey) and II (light grey). Bars represent the standard error (n=4).

Figure 6. Soil extractable C expressed as percentage of total organic carbon (Corg) (a) and soil extractable N as percentage of total nitrogen (TN) (b) across the four pedo-climatic zones: SLU (Nemoral); ORC (Oceanic); AGS (Continental) and UNI (Mediterranean north) in both crop cycles I (dark grey) and II (light grey). Middle line represents the median and whiskers the standard deviations (n=16).

Figure 7. Soil synthetic enzyme index (SEI) (a) and microbial functional diversity (H') (b) across the four pedo-climatic zones: SLU (Nemoral); ORC (Oceanic); AGS (Continental)and UNI (Mediterranean north) in both crop cycles I (dark grey) and II (light grey). Middle line represents the median and whiskers the standard deviations (n=16).

Figure 1

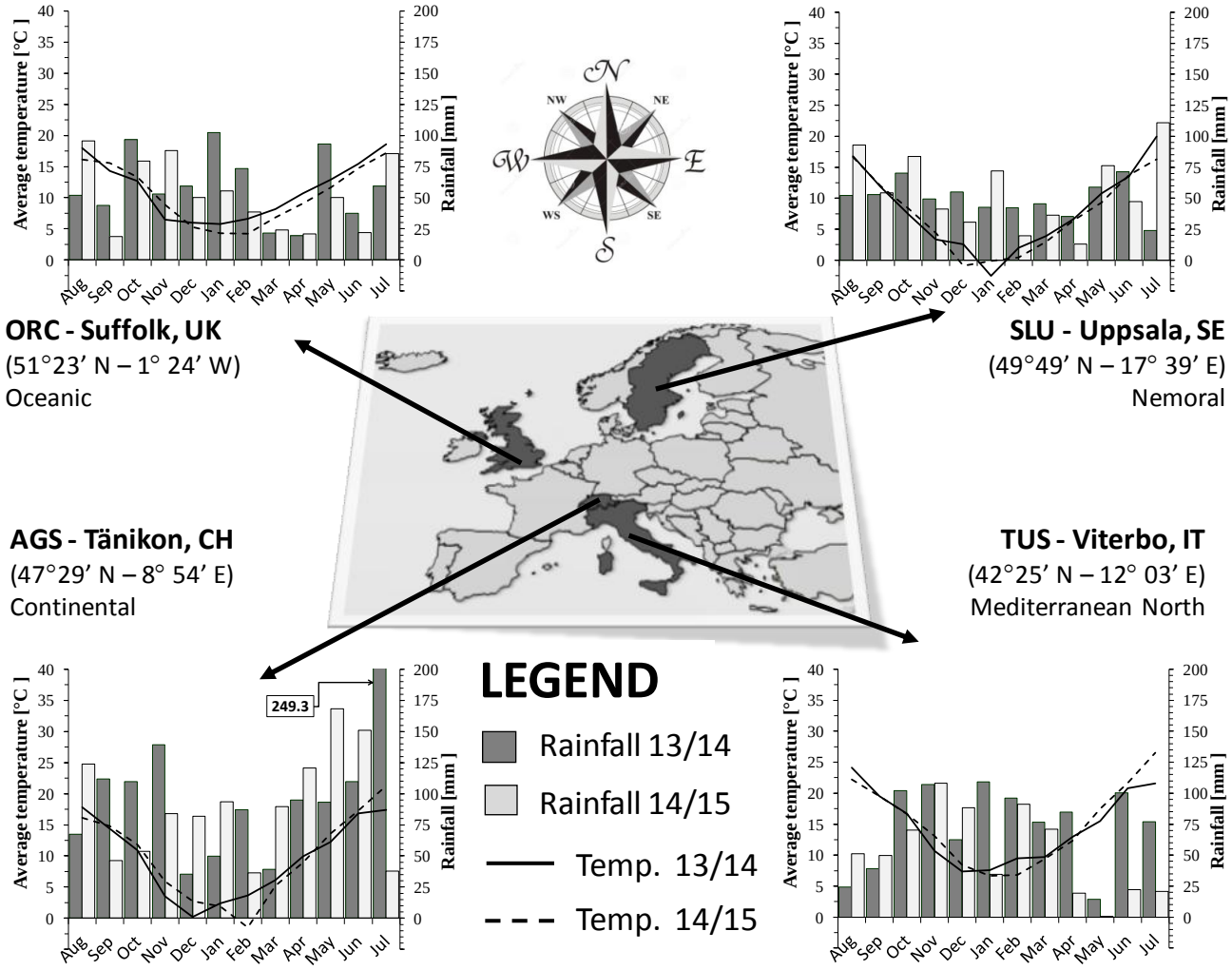

Average temperature [°C]
Rainfall [mm]
Aug Sep Oct Nov Dec Jan Feb Mar Apr May Jun Jul
N
NW
NE
W
E
WS
SE
S
ORC - Suffolk, UK
(51°23' N – 1° 24' W)
Oceanic
SLU - Uppsala, SE
(49°49' N – 17° 39' E)
Nemoral
AGS - Tänikon, CH
(47°29' N – 8° 54' E)
Continental
TUS - Viterbo, IT
(42°25' N – 12° 03' E)
Mediterranean North
249.3
LEGEND
Rainfall 13/14
Rainfall 14/15
Temp. 13/14
Temp. 14/15

Figure 2

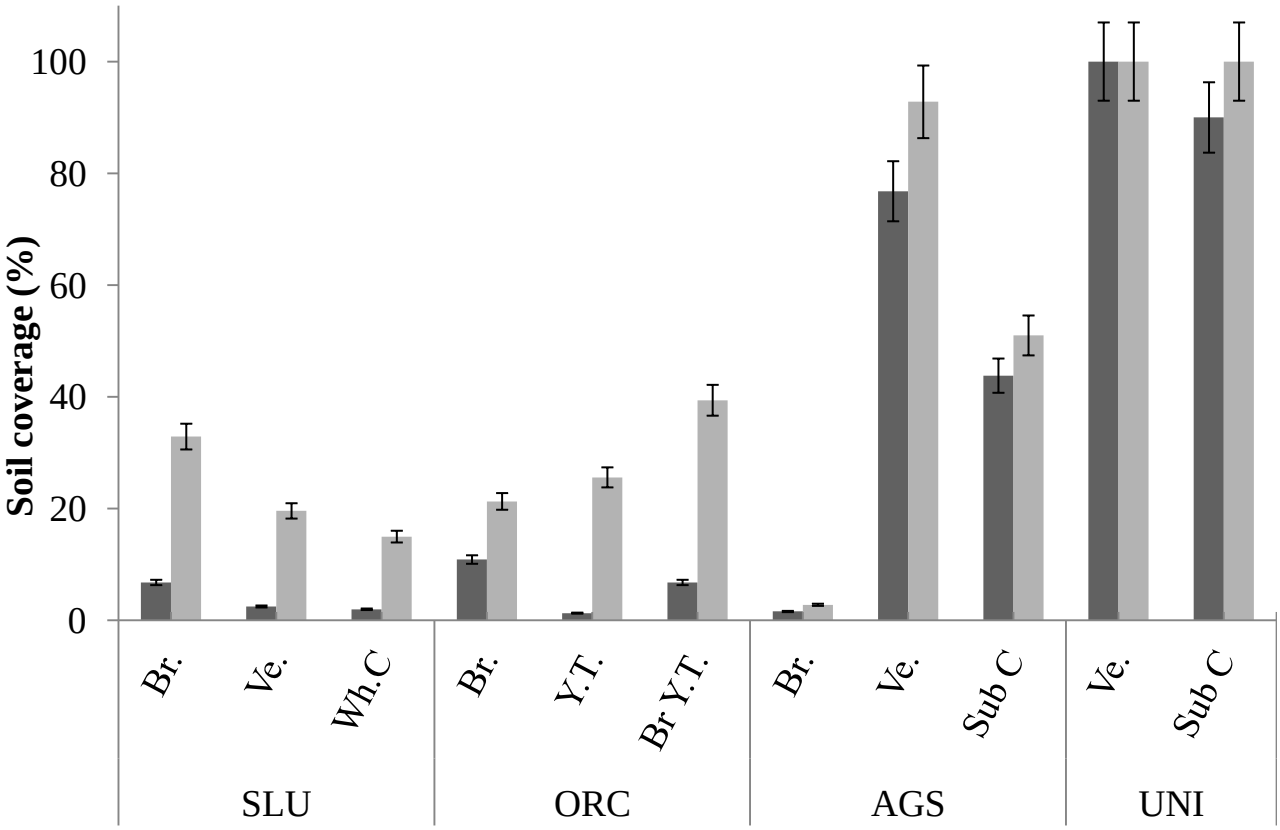

Soil coverage (%)
100
80
60
40
20
0
Br.
Ve.
Wh.C
Br.
Y.T.
Br Y.T.
Br.
Ve.
Sub C
Ve.
Sub C
SLU
ORC
AGS
UNI

Figure 3

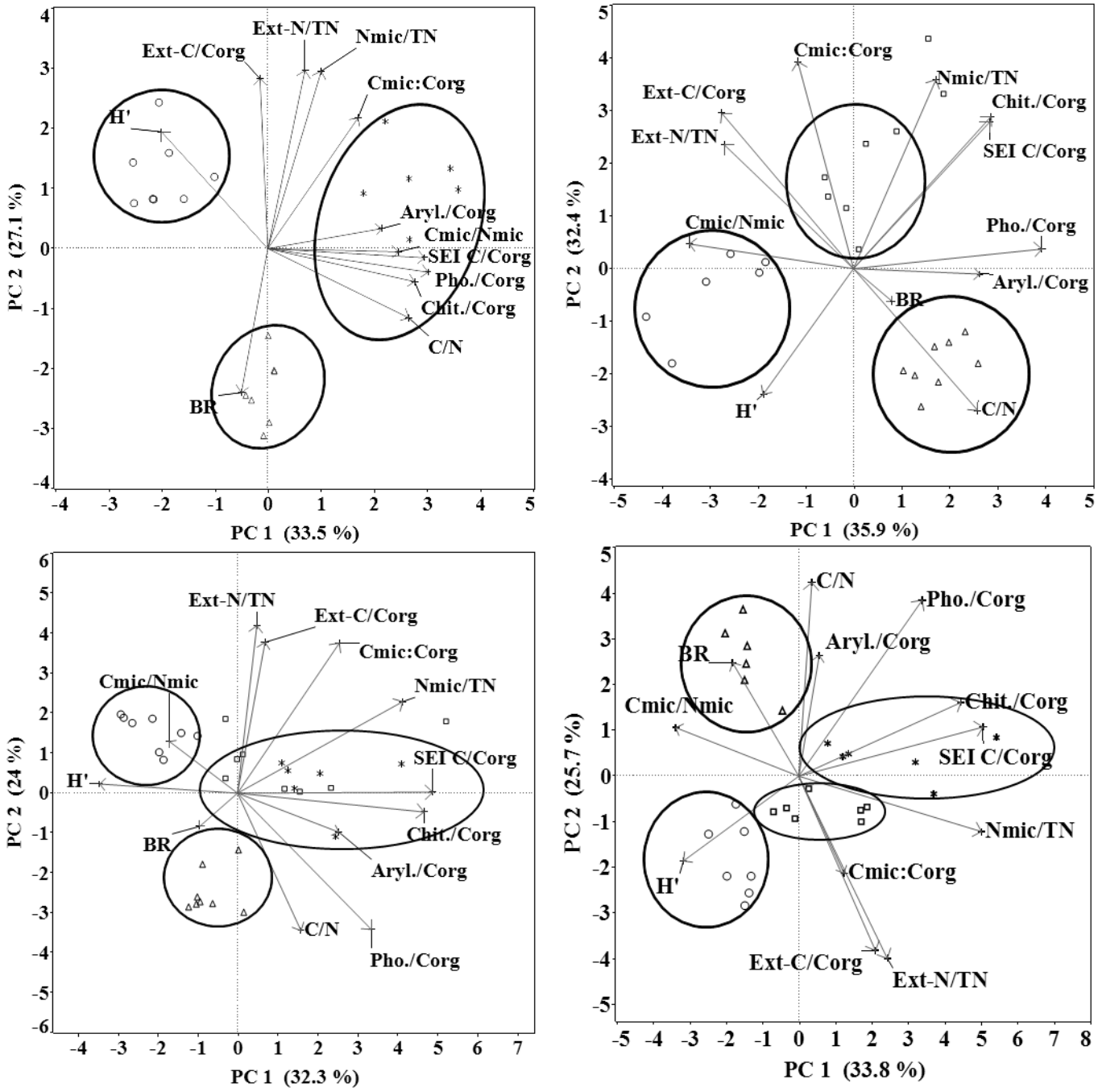

Ext-N/TN
Nmic/TN
Ext-C/Corg
Cmic:Corg
H'
Aryl./Corg
Cmic/Nmic
SEI C/Corg
Pho./Corg
Chit./Corg
C/N
BR
PC 2 (27.1 %)
PC 1 (33.5 %)
Cmic:Corg
Nmic/TN
Ext-C/Corg
Chit./Corg
Ext-N/TN
SEI C/Corg
Cmic/Nmic
Pho./Corg
Aryl./Corg
BR
H'
C/N
PC 2 (32.4 %)
PC 1 (35.9 %)
Ext-N/TN
Ext-C/Corg
Cmic:Corg
Cmic/Nmic
Nmic/TN
SEI C/Corg
H'
Chit./Corg
BR
Aryl./Corg
C/N
Pho./Corg
PC 2 (24 %)
PC 1 (32.3 %)
C/N
Pho./Corg
Aryl./Corg
BR
Cmic/Nmic
Chit./Corg
SEI C/Corg
Nmic/TN
H'
Cmic:Corg
Ext-C/Corg
Ext-N/TN
PC 2 (25.7 %)
PC 1 (33.8 %)

Figure 4

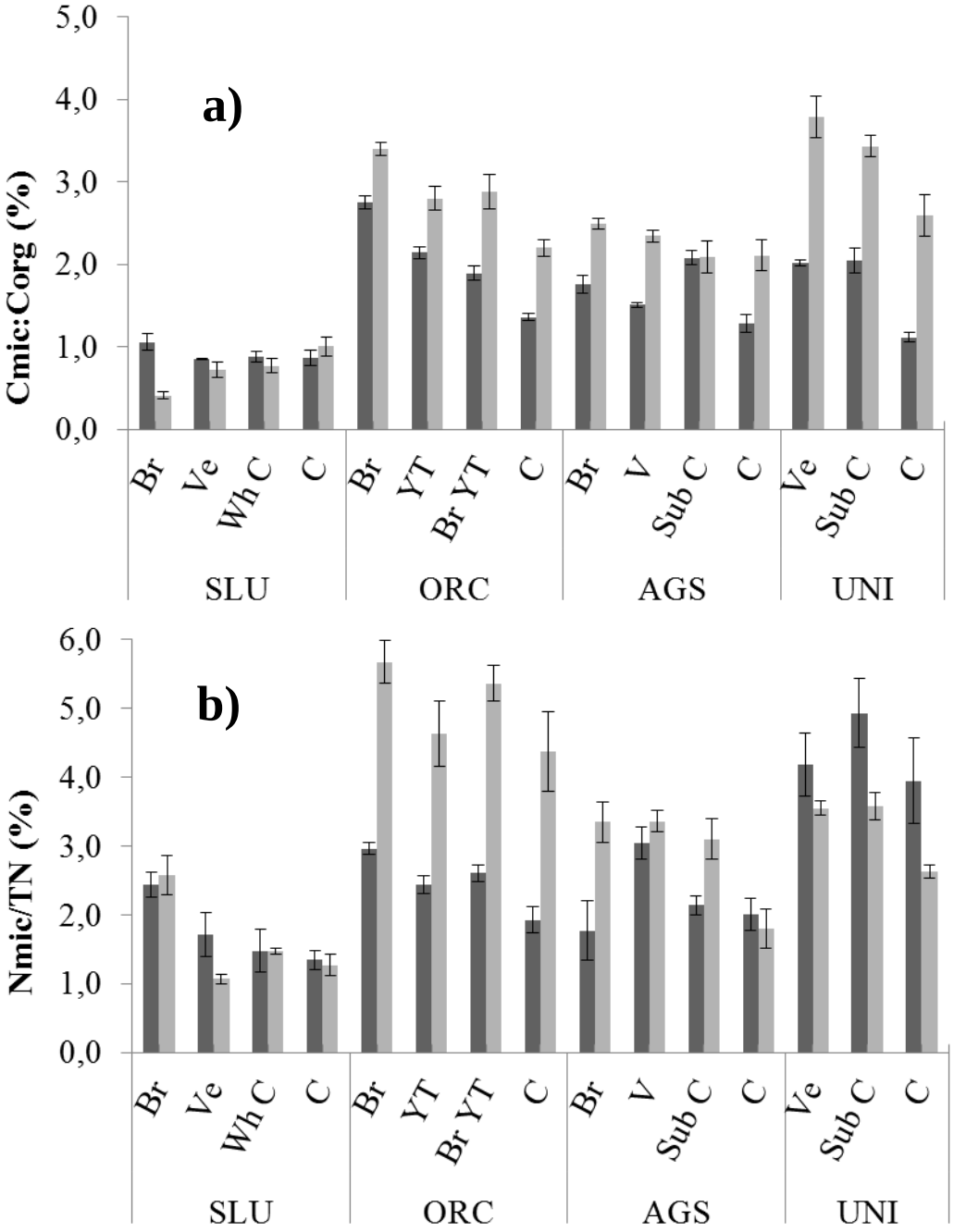

a)
Cmic:Corg (%)
5,0
4,0
3,0
2,0
1,0
0,0
Br
Ve
Wh C
C
Br
YT
Br YT
C
Br
V
Sub C
C
Ve
Sub C
C
SLU
ORC
AGS
UNI
b)
Nmic/TN (%)
6,0
5,0
4,0
3,0
2,0
1,0
0,0
Br
Ve
Wh C
C
Br
YT
Br YT
C
Br
V
Sub C
C
Ve
Sub C
C
SLU
ORC
AGS
UNI

Figure 5

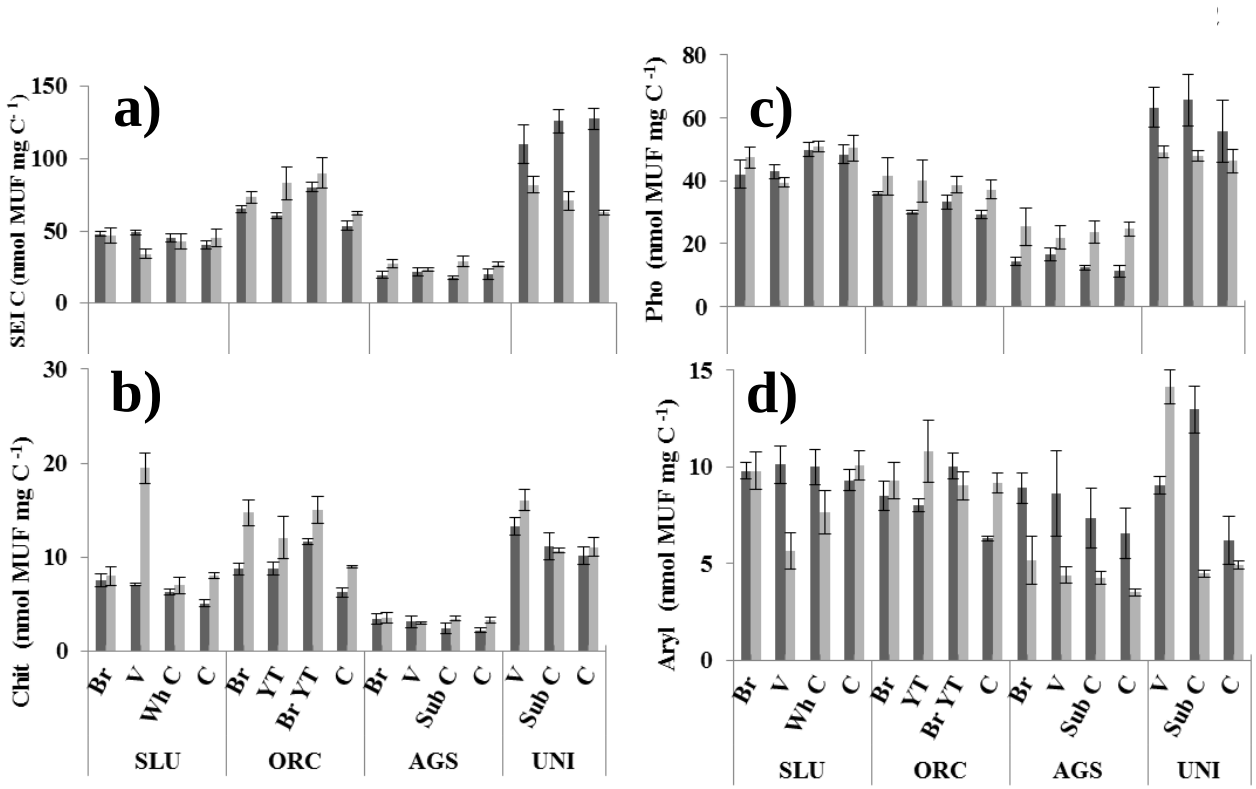

a)
SEI C (nmol MUF mg C -1)
150
100
50
0
b)
Chit (nmol MUF mg C -1)
30
20
10
0
c)
Pho (nmol MUF mg C -1)
80
60
40
20
0
d)
Aryl (nmol MUF mg C -1)
15
10
5
0
Br
V
Wh C
C
Br
YT
Br YT
C
Br
V
Sub C
C
V
Sub C
C
SLU
ORC
AGS
UNI

Figure 6

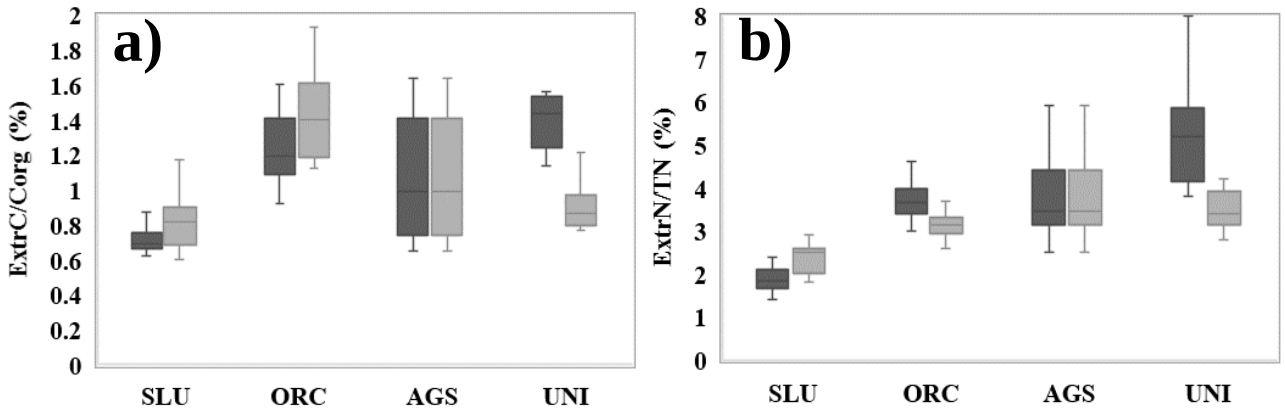
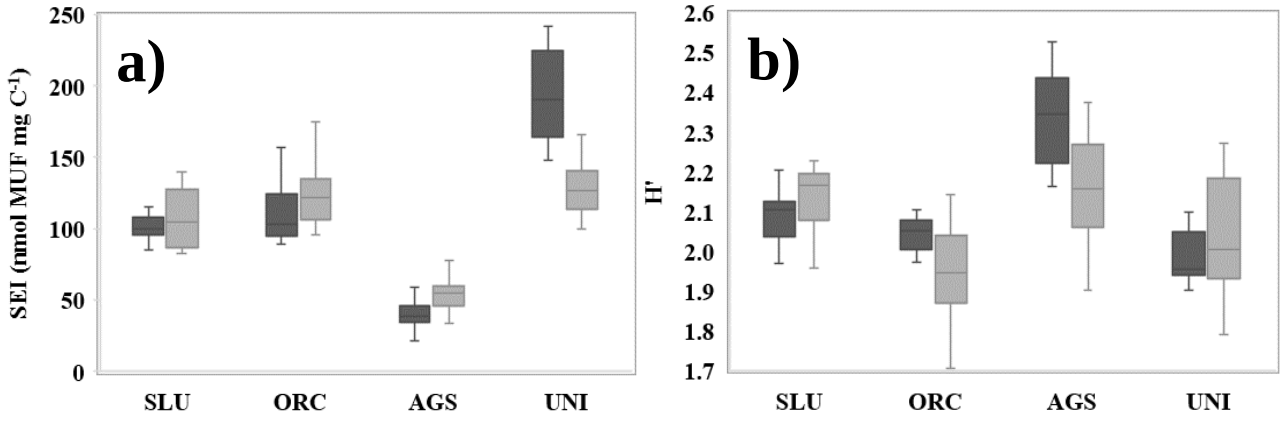

a)
ExtrC/Corg (%)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
SLU
ORC
AGS
UNI
b)
ExtrN/TN (%)
8
7
6
5
4
3
2
1
0
SLU
ORC
AGS
UNI

Figure 7


a)
SEI (nmol MUF mg C-1)
250
200
150
100
50
0
SLU
ORC
AGS
UNI
b)
H'
2.6
2.5
2.4
2.3
2.2
2.1
2.0
1.9
1.8
1.7
SLU
ORC
AGS
UNI