Soil development and microbial functional diversity: Proposal for a methodological approach

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Abstract

The aim of the work was to propose a new methodological approach that relates soil microbial functional diversity to soil development under different moisture regimes. As soil evolution proceeds through an increasing niche separation we expect a link between functional diversity and soil development. Shannon's (H) and Gini (D) diversity indices were calculated using eight enzyme activities (β-cellobiohydrolase, N-acetyl-β-glucosaminidase, β-glucosidase, α-glucosidase, acid phosphatase, arylsulfatase, xylosidase and butyrate esterase) in order to assess functional diversity at different scales, from soil horizons (α-diversity) to soil profiles (β-diversity) under different moisture regime (γ-diversity) and belonging to different taxonomic levels. In addition, the ratio of acid phosphatase to chitinase was calculated as a potential index of soil development. Eight soil profiles were selected: four in Northwestern Italian Alps or Northern Apennines with Udic soil moisture regimes (Typic Haplocryod, Mollic Haplocryept, Lithic Dystrudept, Lithic Cryorthent) and four in Northeastern Italy where in two cases the water table near the soil surface strongly affects the Ustic soil moisture regime, and intrazonal Aquic regime or aquic conditions develop (Typic Haplustept, Typic Ustipsamment, Aquic Ustipsamment, Typic Psammaquent). D ranged from 50 to 95%, while the H ranged from 3 to 2.4 in Lithic Cryorthent and Typic Psammaquent, respectively. Under Udic moisture regime an inverse relationship between soil profile development and the diversity index was observed. However the lowest the diversity in the profile, the highest the variability of the values obtained within horizons in the soil profile suggesting a link between differentiation of soil horizons and biochemical properties. The aquic conditions interfere in establishing the relationship between soil profile development and the microbial functional diversity since H and D increased in Typic Psammaquent with respect to Typic Haplustept (H from 2.4 to 2.6 and D from 52.2 to 60.4). Finally, the phosphatase/chitinase ratio was related to soil development since the lowest values were obtained in the upper horizons of Typic Haplustept, Typic and Aquic Ustipsamment (from 2.0 to 4.0), while the highest values were obtained in deep horizons of Typic Haplocryod and Lithic Dystrudept (e.g. 39.2 in Bs2 and 28.0 in Bw1). In conclusion, microbial functional

diversity assessed using Shannon or Gini diversity indices and phosphatase/chitinase ratio measured at different scales from soil horizons to soil profiles under different moisture regime and belonging to different taxonomic levels, may represent a new approach to establish the interrelationship between pedogenetic processes, soil development and soil microbial functions.

Introduction

To date ecological theories have been based mainly on the study of aboveground ecosystems. Despite the fact that the soil biota play a fundamental role in ecosystem functioning, through nutrient cycling, decomposition and energy flow, soil organisms have had a negligible influence on the development of contemporary ecological theories (Wardle and Giller, 1996).

In the last two decades the interest about soil biodiversity and ecosystem functioning has become more and more important in ecological sciences. Both community structure and metabolic functions can be taken into account to evaluate soil microbial biodiversity and both are essential in the evaluation of the role that microorganisms play in determining soil quality. Microbial functional diversity is defined, operationally, as the numbers, types, activities, and rates at which a suite of substrates is utilized by the bacterial community (Zak et al., 1994). It can also be described through the composition of microbial communities needed to perform and maintain ecosystem processes in the soil such as decomposition and mineralization.

The functional diversity results from genetic variability within a taxon, environmental effects on gene expression and ecological interactions among taxa. Zak et al. (1994) point out that there is a general lack of information relating taxonomic diversity to ecosystem functions, so "functional rather than taxonomic diversity may provide greater insight to microbial roles in ecosystems."

If the molecular techniques measure the diversity of the numerically dominant members of the community (Lynch et al., 2004), functional diversity is related to the activity of the soil and gives information on the functioning of those members of the soil microflora involved in specific processes. Distinct from the genetic diversity of the soil microbial biomass (Wellington et al., 2003; Emmerling et al., 2002; Kennedy and Grewin, 1997; Zak et al., 1994) which assesses the potential of the community for enzyme synthesis, functional diversity is thus related to the actual activities resulting from that potential. The actual rate of enzyme production and the fate of produced enzymes are in fact modified by environmental effects as well as by ecological interactions (Kandeler et al., 1996). Also of importance is the opportunity to use enzyme activities or sole-carbon-source utilization patterns data to assess microbial diversity with measures such as the Shannon index (Zack et al., 1994; Staddon et

al., 1997) and the Gini diversity coefficient (Harch et al., 1997). In soil ecology these indices offers the potential to monitor changes in microbial diversity caused by environmental changes, land-management, and pollution (Ipsilantis and Coyne, 2007; Tribedi and Sil, 2012; Marinari et al., 2012).

In the assessment of soil microbial functional diversity, it is important to note that soil microorganisms are characterised by a spatial diversity with possible differences between rhizosphere and bulk soil, macroaggregates and microaggergates, macropores and micropores, different horizons, etc. (Lynch et al., 2004). Typically, well developed soils are largely stratified habitats, with distinct horizons; each of them may be regarded as a separate entity in that it differs in physical and chemical properties. At the beginning of soil formation the anisotropy of the profile is low, and well expressed soil horizons are lacking, but with the ongoing of pedogenesis varying physical and chemical environments differentiate. Functional diversity of soil enzymes is tightly linked to biogeochemical cycles and thus should reflect the changing fertility conditions which occur during soil development. Olander and Vitousek (2000) were able to evidence an increase in phophatase with respect to N-acetylglucosamidase with increasing age in a soil chronosequence, testifying the well known shift from N- to P-limitation with increasing soil development (Wardle et al., 2004). Besides time of soil formation, in humid and temperate well drained environments, profile differentiation is mainly driven by water flowing through the profile, reacting with the solid phase and leaching cations out of the solum (e.g. Chesworth, 1992). The amount of water passing through the profile, hence available for profile horizonation is most directly linked to climate, as it depends on rainfall and temperature that regulates evaporation. When large amounts of water are coupled with highly acidifying vegetation, the effects on profile development are striking and well differentiated soil horizons originate, as in Spodosols (e.g. Lundström et al., 2000). Water availability for pedogenic processes can however be modified by local factors, particularly in non extreme environments; the presence of an excess water, caused for example by the presence of a superficial water table, deeply influences the dynamics of several elements and dramatic changes in soil gases partial pressures may greatly affect spatial diversity of soil organisms through the profile (Skoop et al., 1990; Schütz et al., 2010).

How the diversity of these soil habitats can be incorporated in a general soil microbial diversity concept is not known. Niche separation is an important mechanism to increase soil biodiversity, and the most striking niche differentiation in soil is visible when the whole profile is considered. On a landscape level, biodiversity may be viewed at different levels of

resolution. Whittaker (1972) proposed to distinguish between diversity of species within a community of a habitat (α-diversity), rate and extent in change of species along a gradient of habitats (β-diversity) and richness of species over a range of habitats (γ-diversity). This concept, plausible for traditional habitat diversity, may also be used to describe soil microbial diversity concepts. Moreover, among numerous factors that are known to affect biodiversity (e.g. trophic interactions, spatial and temporal habitat heterogeneity), increased stability, resilience, resistance to stress, and even productivity may have positive effects on biodiversity (Griffiths et al., 1997; Nannipieri et al., 2003).

The aim of the work was to propose a new methodological approach that relates soil microbial functional diversity to soil development under different moisture regimes. We therefore analysed eight enzyme activities in order to assess functional diversity at different scales, from soil horizons (α-diversity) to soil profiles (β-diversity) under different moisture regime (γ-diversity) and belonging to different taxonomic levels. As soil evolution proceeds through an increasing niche separation we expect a link between functional diversity and soil development.

Material and methods

Site and soil descriptions

To take into account both climatic factors influencing soil organisms and water availability for profile horizonation we selected 8 soils in northern Italy (Figure 1) with two contrasting dominant soil moisture regimes: Udic and Ustic. The Udic moisture regime is common to the soils of humid climates that have well distributed rainfall. According to the USDA Soil Taxonomy (Soil Survey Staff, 1999) in these soils the moisture section is dry for less than 90 cumulative days. Instead, in the Ustic regime, the soil moisture is limited but is present at a time when conditions are suitable for plant growth, and the soil control section is dry for more than 90 cumulative days in a normal year. The soil moisture regimes were calculated using the Newhall Simulation Model (Van Wambeke et al., 1986). The Udic selected soils were from Northwestern Italian Alps or Northern Apennines, and ranged in development from Entisols to Inceptisols and Spodosols (Table 1). The Ustic soils were all sampled in the same geographic area, a coastal plain in Northeastern Italy, they ranged from Entisols to Inceptisols. In this area, the water table near the soil surface strongly affects the soil moisture regime, and intrazonal Aquic regime or Aquic conditions (Typic Psammaquent and Aquic Ustipsamment, respectively) occur in the interdunal depressions, where therefore water saturation is present (Marinari et al., 2012).

Soil chemical properties

Soil samples were taken from each genetic horizon, air dried, ground by hand and passed through a 2 mm sieve. Physicochemical characterisations were carried out on the fine earth fraction and the data were reported in Table 2. The pH was determined potentiometrically using a 1:2.5 soil:deionised water ratio; the particle size distribution was obtained by the pipette method; total N was determined by the Kjeldhal method (Bremner and Mulvaney, 1982); total organic carbon (C_{org}), was analysed by wet oxidation at 160 °C with $K_2Cr_2O_7$ 1/3 M, according to the method of Springer and Klee (1954). The total extractable carbon (C_{extr}) was extracted using a solution of NaOH 0.1 M and $Na_4P_2O_7$ 0.1 M incubated at 65 °C for 24 h and the C concentration in the extracts was determined by wet-oxidation.

Enzyme activities

Enzyme activity was measured according to the methods of Marx et al. (2001) and Vepsalainen et al. (2001), based on the use of fluorogenic methylumbelliferyl (MUF)-substrates. Soils were analysed for β-cellobiohydrolase (EC 3.2.1.91), N-acetyl-β-glucosaminidase (EC 3.2.1.30), β-glucosidase (EC 3.2.1.21), α-glucosidase (EC 3.2.1.20), acid phosphatase (AP, EC 3.1.3.2), arylsulfatase (EC 3.1.6.1), xylosidase (EC 3.2.2.27) and butyrate esterase (EC 3.1.1.1) using 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, respectively. A moist sample (equivalent weight to 1 g oven-dry material) was weighed into a sterile jar and 50 ml of Na-acetate buffer pH 5.5 were added. A homogenous suspension was obtained by homogenising with UltraTurrax at 9600 rev min^{-1} for 3 min. Aliquots of 100 µl were withdrawn and dispensed into a 96 well microplate (three analytical replicates $sample^{-1}$ $substrate^{-1}$). Finally, 100 µl of 1 mM substrate solution were added giving a final substrate concentration of 500 µM. Fluorescence was measured after 0, 30, 60, 120, 180 min of incubation at 30 °C. Fluorescence (excitation 360 nm; emission 450 nm) was measured with an automated fluorimetric plate-reader (Fluoroskan Ascent).

The enzyme specific activity (per unit of C_{org}) was calculated in order to keep the amount of organic matter as an internal control (Trasar-Cepeda et al., 2008). Moreover, C horizons were not considered being C_{org} content negligible.

The ratio of acid phosphatase (which mineralizes P) to chitinase (N-acetyl-β-D glucosaminide) was calculated as a potential index to link enzyme activity to soil development (Caldwell et al., 2005).

Functional diversity indices

From the values of the eight enzyme activities, soil functional diversity was determined using the Shannon's diversity index calculated by equation (1) (Bending et al., 2002)

$$H' = -\sum p_i \log_2 p_i \quad (1)$$

where p_i is the ratio of the activity of a particular enzyme to the sum of activities of all enzymes. The Gini's concentration index, calculated using the following equation (Harch et al., 1997; Sharma et al., 1998), was also taken into account:

$$G = \frac{N+1}{N} - \frac{2}{N}\sum_{i=1}^{N} q_i \quad (2)$$

where qi refers to the cumulative frequency value of enzyme activities of each soil horizon, N to the total number of enzymes activities tested.

The minimum value of the Gini's concentration index is 0 when the activity of all enzymes within a soil horizon is the same, and the theoretical maximum is 1, when the activity of all enzymes except one is 0. The Gini's diversity index, named D, is expressed by the complement of Gini's concentration index i.e. $D = (1-G)\cdot 100$. Thus higher values of H' and D higher mean indicate higher microbial functional diversity level.

The order of magnitude of the values obtained for the different enzyme activities varies considerably depending on the single one being determined, thus leading to some enzyme activities having more weight than others. To solve this problem, the percentage of single enzyme activity with respect to its total activity along soil profile (sum of values obtained in each horizons) was used instead of the percentage of the maximum value obtained in a range of soils (Marinari et al., 2012) for the calculation of both diversity indices, H' and D.

The two indices described above were calculated for each horizon (α-diversity), while the final value for each profile was obtained calculating the average, weighed for each horizon thickness, along soil profile (β-diversity).

Results

Soil specific enzyme activities

The specific activity (per unit of C_{org}) of the enzymes involved in carbon cycle under Udic regime decreased along soil profile and according to soil development level (Figure 2). The

trend of β-glucosidase specific activity overlapped the global one (Figure 3). Also the cellulase specific activity showed a quite similar trend (Figure 4), with the exception of the Spodosol where it decreased in the E horizon and accumulated in the EB and Bs horizons. Under Ustic regime the specific activities were higher in Typic Ustipsamment than in Typic Haplustept, but where aquic conditions occurred the specific enzyme activities decreased (Figure 2). A reduction of the specific enzyme activity was thus observed both with soil development and with increasing water saturation.

The lowest values of the phosphatase to chitinase ratio were obtained in the upper horizons of soils under Ustic moisture regime (from 2.0 to 4.0), while the highest values were in deep horizons of soils under Udic moisture regime (e.g. 39.2 in Bs2 of the Typic Haplocryod and 28.0 in Bw1 of Lithic Dystrudept) (Figure 5). In general, the two soils showing the highest ratios were those with acidic pH (Table 1).

Functional diversity indices

Gini diversity index, measured in the eight soil profiles is showed in figure 6 and ranged from 50 to 95%. The highest value was observed in the Lithic Cryorthent, while the lowest in the Typic Psammaquent. Under Udic moisture regime an inverse relationship between soil profile development and the diversity index was observed; however the lowest the diversity in the profile, the highest the variability of the values obtained within horizons in the soil profile. A similar trend was observed under Ustic soil moisture since the Haplustept showed lower diversity index and higher horizon variability than Ustipsamments. Aquic conditions interfere in the relationship between soil profile development and the diversity index since the Typic Psammaquent had the lowest diversity index and high variability within horizons. The Shannon's diversity index is showed in Figure 7 ranging from 3 for Lithic Cryorthent to 2.4 in Typic Psammaquent.

Gini's coefficient was related to Shannon diversity index by a logarithmic function (Figure 8). Conversely, Gini's coefficient was inversely related to C:N ratio as shown in Figure 9.

Discussion

This study aimed to introduce for the first time, to our knowledge, the use of microbial functional diversity index within a soil characterization procedure. Few examples in the recent literature introduced biochemical properties with the aim to describe soil profile or to discuss the influence of soil forming factors (e.g. lithological substrate) on soil biological activity (Marinari and Vittori Antisari, 2010). Enzyme activities might be studied as specific

activity (per unit of Corg) when the aim of the study is to compare biochemical properties in soils with significant differences in organic matter content (Trasar-Cepeda et al., 2008) and particularly where soil horizons were considered. In our study the specific activity of enzymes involved in carbon cycle was the lowest in deep horizons of Spodosol, suggesting a reduction of hydrolizable organic substrates in the deep horizons of developed soils (e.g. Bs in Typic Haplocryod). Moreover, the particular trend observed in the Spodosol for cellulase activity, showing a decrease in the E horizon and an increase in the B horizon, reflects the pedogenic processes such as eluviation/illuviation of lignocellulosic material (Guggenberger et al., 1994; Wilcken et al., 1997). This may in fact suggest an accumulation of cellulosic material coming from fresh organic matter degraded in surface horizons and accumulated in the Bs through possible interactions with the mineral fraction (Guggenberger and Zech, 1994).

The trend of β-glucosidase specific activity overlapped the one observed for the four enzymes involved in carbon cycle, confirming its role as a keystone and reliable biochemical indicator of changes in soil carbon cycle (Knight and Dick, 2004; Lagomarsino et al., 2009).

Acid phosphatase enzymes are the dominant group of enzymes involved in mineralizing P. They cleave ester bonds in a variety of organic phosphorus compounds, and release phosphate (Malcolm 1983; Duff et al., 1994). Chitinases are essential enzymes in the main pathway of chitin degradation. They hydrolyze the glucosidic bonds of chitin releasing a smaller N containing organic compound. A common fate of this compound is mineralization to inorganic N (Gooday 1994). Both phosphatase and chitinase are produced by plants, fungi, and bacteria, with fungi being the dominant chitinase producers in soils (Gooday, 1994). A relationship between phosphatase to chitinase ratio and soil age has been observed in previous studies (Olander and Vitousek, 2000) mainly because of the decrease of chitinase activity across the chronosequence, reflecting increasing N supply. In this study the obtained results were consistent with values reported in previous work (Olander and Vitousek, 2000; Caldwell, 2005), where in modern, 300-year-old soil, the phosphatase to chitinase ratios in the mineral soil horizons was 2.85. For 20,000-year-old soil, the ratios increased to 18.6, suggesting an increasing importance of organic phosphorus, relative to organic nitrogen, with soil development. In our work, the increase of this ratio was mainly due to the enrichment of phosphatase activity, and two explanations are possible. The increase may be promoted by the enzyme protection through their immobilization and accumulation on clay surface, humic substances and organo-mineral complexes (Rao et al., 2000). For this reason the highest value of phosphatase/chitinase ratio in the Bs2 horizons of Typic Haplocryod may also suggest a higher stability of phosphatase to proteolytic attack due to the interaction of this enzyme with

organo-mineral complexes (Rao and Gianfreda, 2000). On the other hand, the highest phosphatase activity in the most acidic soils may be the result of the increase in substrate availability; phosphorus pools shift from easily soluble phosphatic minerals such as apatites (e.g. Eger et al., 2011), to organic phosphates that govern P availability in more leached acidic soils (Tiessen et al., 1984).

The Shannon's diversity index did not help to clearly individuate a trend with soil moisture regimes and profile development. The two diversity indexes were well related to each other, but Shannon's H was less effective in discrimination, particularly in the case of high diversity. This could probably be ascribed to the logarithmic transformation required by H' calculation that levels out the differences with increasing index values. On the other hand the calculation of Gini diversity index allowed a clear discrimination among the eight selected soils profiles. In particular, under Udic moisture regime an inverse relationship between soil profile development and the microbial functional diversity index of soil profile (β-diversity) was observed. In addition the lowest the diversity index of soil profile the highest the variability of the values obtained in the horizons (α-diversity). This large range α-diversity could be related to the differentiation of niches with specific chemical biochemical and physical characteristics. In Spodosols in fact, relatively organic matter rich A horizons are followed by carbon and iron depleted eluvial ones, followed in turn by illuvial horizons where selected compounds accumulate as organo-mineral complexes (e.g. Sauer et al., 2007). Even at a relatively lower stage of soil development, as in Inceptisols, the differentiation of horizons occurs through weathering of primary minerals, formation of soil silicates and iron (hydr)oxides, structure development and movements of base cations through the soil profile (Bockheim and Gennadyiev, 2000). Consequently, various levels of protection of organic matter within aggregates and differences in the availability of phosphorus are expected and may influence biodiversity through the creation of specific niches. Conversely, in Entisols, profile differentiation is weak and mainly depends on organic matter, hence in good agreement with the lowest variability in biodiversity indexes in the least developed soil. The inverse relationships observed between C:N ratio and Gini's coefficient may suggest that niches with organic matter showing a C:N ratio in the range 10-15 are linked to the highest functional diversity values.

According to the literature, higher evenness usually reflects higher functionality and stability within a system (Legendre and Legendre, 1998). In this work we introduce the hypothesis that links microbial functional diversity level to the highest soil entropy. In fact, the initial step of pedogenesis corresponds to the highest entropy level of the soil system according to the

"energy" soil development model (reviewed e.g. by Minasky et al., 2008), and there a wide array of substrates, chemical and biochemical processes are found in the whole profile. Soil entropy then reduces due to flow to external environment in an open system (Smeck et al., 1983), horizons differentiate and functional biodiversity decreases as well. Shannon diversity index is also related to the information entropy of a system and when applied as a measure of microbial hydrolitic functions entropy, may express the order of soil organic substrates availability, linked lead not only to environmental factors but also to soil pedogenic processes.

Aquic regime could interfere with the above described relationship between soil profile development and the functional diversity index. The Aquic moisture regime is a reducing regime in a soil that is virtually free of dissolved oxygen because it is saturated by water (Soil Survey Staff, 1999), that may sharply affect microbial functions. In our study, the presence of a water table near to the soil surface in the Typic Psammaquent determines the lowest profile functional diversity. It is known that water content exerts a strong control on soil biogeochemistry, including biogeochemical cycling of nitrogen and carbon (Gower et al., 1992; Porporato et al., 2003; Ridolfi et al., 2003; Turcu et al., 2005). Focusing on the soil microbial population as the powerhouse of biogeochemical reaction, Skoop et al. (1990) suggested that aerobic microbial activity is constrained by diffusion limiting processes. In a previous work Schütz et al. (2010) found that in a not water saturated site, specific enzyme activities were significantly associated with the community composition, whereas at the water saturated sites they were not. The strong correlation between structure and function at the well drained site reflects the lack of external nutrient input and the resulting need to gain this by degrading complex organic substrates through soil enzymes. In contrast, the microbial community at the saturated sites is fed with readily available nutrients (e.g. soluble organic substrates and NO_3^-) without the necessity for the production of a perfect matching and complex set of soil enzymes. Hence, groundwater recharge conditions not only uncoupled catabolism from growth, but presumably also organic matter breakdown from soil enzymes (Schütz et al., 2010).

Finally, since the highest functional diversity values have been found in horizons with a C:N ratio typical of highly humified organic matter (10-15), we can hypothesize that conditions favouring humification process can, at the same time, promote microbial functional diversity as the significant Person correlation coefficient between Gini coefficient and total organic carbon or extractable carbon ($r=0.551$ and $r=0.591$ respectively, $p< 0.01$) demonstrate.

Conclusion

The phosphatase/chitinase ratio and microbial functional diversity measured using Shannon or Gini diversity indices at different scales from soil horizons (α-diversity) to soil profiles (β-diversity) under different moisture regimes and belonging to different taxonomic levels, may represent a new approach to establish the interrelationship between pedogenic processes, soil development and soil microbial functions. In particular, although Aquic regime interfered with the relationship between soil profile development and the microbial functional diversity index, under Udic moisture regime an inverse relationship was found. Therefore, soil microbial functional diversity, measured using Shannon and Gini diversity indices, might be considered linked to the highest entropy of young undeveloped soils.

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Table 1: Soil site description and classification

Profile (N°)	Elevation (m a.s.l.)	Dominant soil temperature regime	Dominant soil moisture regime	Dominant vegetation	Horizon sequence	Solum depth (cm)	USDA Soil Classification
1	1825	Cryic	Udic	Larch	A-E-EB-Bs1-Bs2-Bs3-C	64	Typic Haplocryod
2*	1620	Cryic	Udic	Pasture with larches	A-Bw-C	40	Mollic Haplocryept
3	1681	Mesic	Udic	Conifer stand	AB-Bw1-Bw2-C	32	Lithic Dystrudept
4	1729	Frigid	Udic	Mountain pasture	A1-A2-AC-C	20	Lithic Cryorthent
5	2.0	Mesic	Ustic	Pinewood	A1-A2-Bw-C	80	Typic Haplustept
6**	1.5	Mesic	Ustic	Maritime pines with brushwood	A-AC-C	23	Typic Ustipsamment
7**	1.0	Mesic	Ustic (Aquic[¥])	Evergreen oaks with wet association	A1-A2-AC-Cg1-Cg2	60	Aquic Ustipsamment
8*	0.5	Mesic	Aquic	Swamp forest	A1-A2-Cg1-Cg2	20	Typic Psammaquent

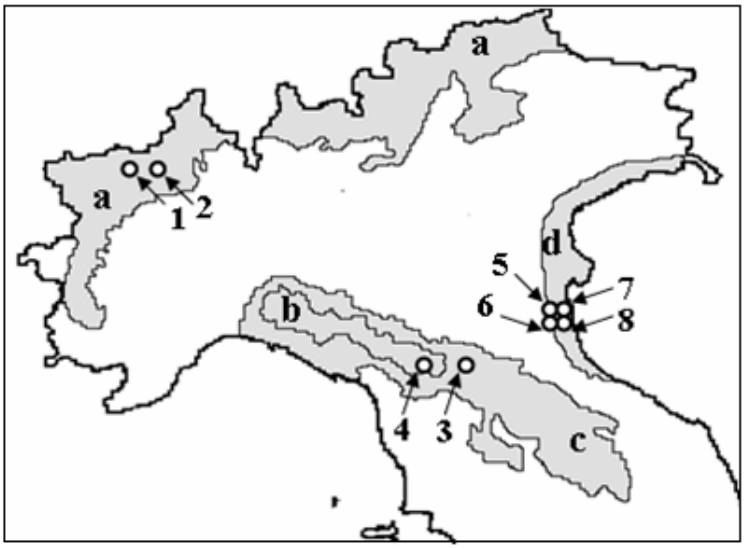
*Described in previous works: Scalenghe et al. (2002); **Marinari et al. (2012)
[¥] aquic conditions occurred below 60 cm

Table 2: Soil physicochemical properties

Profile (N°)	Horizons	pH(H_2O)	$CaCO_3$	Corg	TN	C:N	C_{ext}	Clay	Silt	Sand
			g kg^{-1}	g kg^{-1}			g kg^{-1}		%	
1	A	4.6	nd	49.7	2.7	18.4	34.6	1.8	12.7	85.5
	E	4.7	nd	14.0	0.6	23.3	6.6	4.2	17.6	78.2
	EB	4.6	nd	12.1	0.4	30.3	6.8	3.9	15.4	80.7
	Bs1	4.9	nd	11.2	0.4	28.0	6.5	3.2	11.4	85.4
	Bs2	5.0	nd	13.3	0.5	26.6	6.9	2.4	10.2	87.5
	Bs3	5.1	nd	14.4	0.5	28.8	6.7	0.9	8.3	90.7
	C	5.5	nd	3.7	0.1	37.0	nd	0.7	6.6	92.7
2*	A	6.5	0	63.0	5.3	11.9	nd	3.0	31.0	66.0
	Bw	7.1	0	27.0	3.0	9.0	nd	2.0	24.0	74.0
	C	8.1	20	4.0	0.6	6.7	nd	2.0	25.0	73.0
3	AB	4.4	nd	65.0	5.0	13.0	34.4	4.8	27.9	67.3
	Bw1	4.7	nd	35.0	3.0	12.0	19.7	5.7	25.2	69.1
	Bw2	4.9	nd	21.0	2.0	9.0	12.3	15.9	39.2	44.9
	C	5.1	nd	7.0	<0.1	nd	nd	7.5	32.7	59.8
4	A1	6.5	<0.2	113.0	7.0	16	nd	1.4	18.5	80.1
	A2	6.8	<0.2	39.0	4.0	10.0	24.1	1.2	18.9	79.9
	AC	7.1	<0.2	34.0	3.0	10.0	18.3	3.2	22.1	74.7
5	A1	5.8	0	139.8	6.9	20.1	75.3	0.2	17.2	82.6
	A2	7.2	18	46.5	3.0	15.6	29.2	0.1	9.8	90.1
	Bw	8.4	123	4.2	0.2	24.5	0.5	nd	nd	nd
	C	8.5	123	7.0	0.2	30.3	nd	nd	nd	nd
6**	A	6.4	7	52.3	3.0	17.4	23.7	4.2	5.3	90.5
	AC	8.0	20	5.0	0.6	8.3	3.2	3.0	5.4	91.6
	C	8.5	56	1.1	0.1	11.0	nd	2.0	3.1	94.9
7**	A1	7.4	20	31.9	3.0	10.6	20.3	5.0	12.8	82.2
	A2	7.8	18	16.9	1.2	14.1	7.5	2.3	8.7	89.0
	AC	7.9	25	11.0	1.0	11.0	7.5	2.9	3.5	93.6
	Cg1	8.1	61	2.3	0.2	11.5	Nd	2.9	5.4	91.7
	Cg2	8.3	82	1.7	0.2	8.3	nd	nd	nd	nd
8*	A1	7.1	26	71.2	5.5	12.9	17.0	7.2	6.7	86.1
	A2	7.6	13	36.6	2.4	15.3	16.9	2.9	8.9	88.2
	Cg1	8.3	85	1.2	0.7	1.7	1.45	1.6	5.4	93.0
	Cg2	8.2	60	1.7	0.1	16.5	nd	1.0	1.6	97.4

*Described in previous works: Scalenghe et al. (2002); **Marinari et al. (2012)
$CaCO_3$: total carbonate; Corg: total organic carbon;TN: total nitrogen; C_{ext}: extractable carbon
nd: not detected

Figure 1: Soil sampling sites according to Italian Soil Regions.



Soil regions

a	Western and central Alps on igneous and metamorphic rocks	c	Northern and central Apennine
b	Highest part of northern Apennine	d	Coastal plains of northern Italy

Soil profile	WGS84-UTM	
	E (m)	N (m)
1	409189 E32	5066788
2	408616 E32	5074727
3	646498 E32	4888068
4	646634 E32	4888022
5	280209 E33	4928509
6	280023 E33	4933960
7	280655 E33	4933957
8	279845 E33	4934006

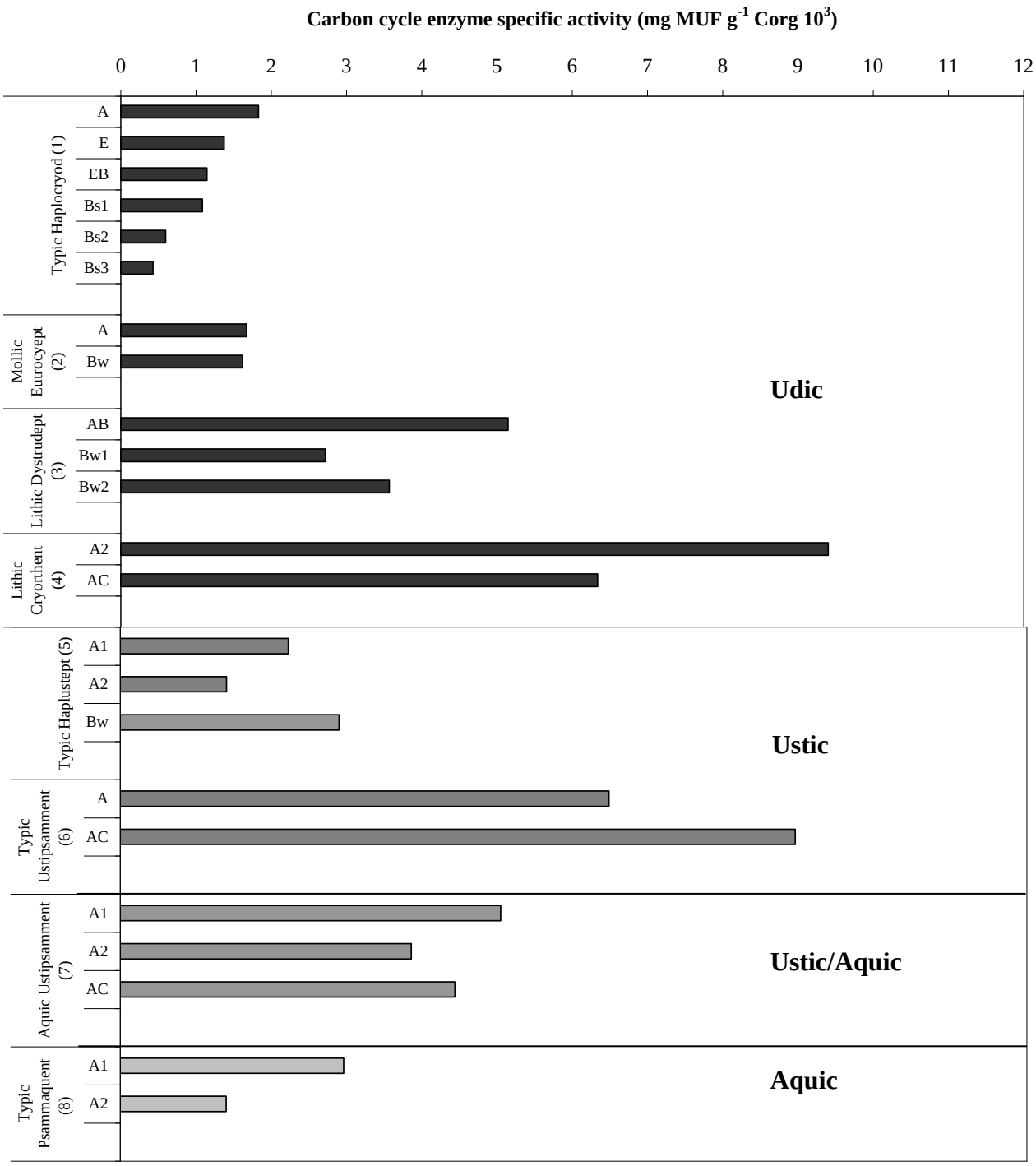


Figure 2: Specific activity (per unit of organic carbon) of four enzymes involved in carbon cycle (cellulase, β-glucosidase, α-glucosidase, xylosidase) along soil profiles under Udic, Ustic and Aquic soil moisture regimes

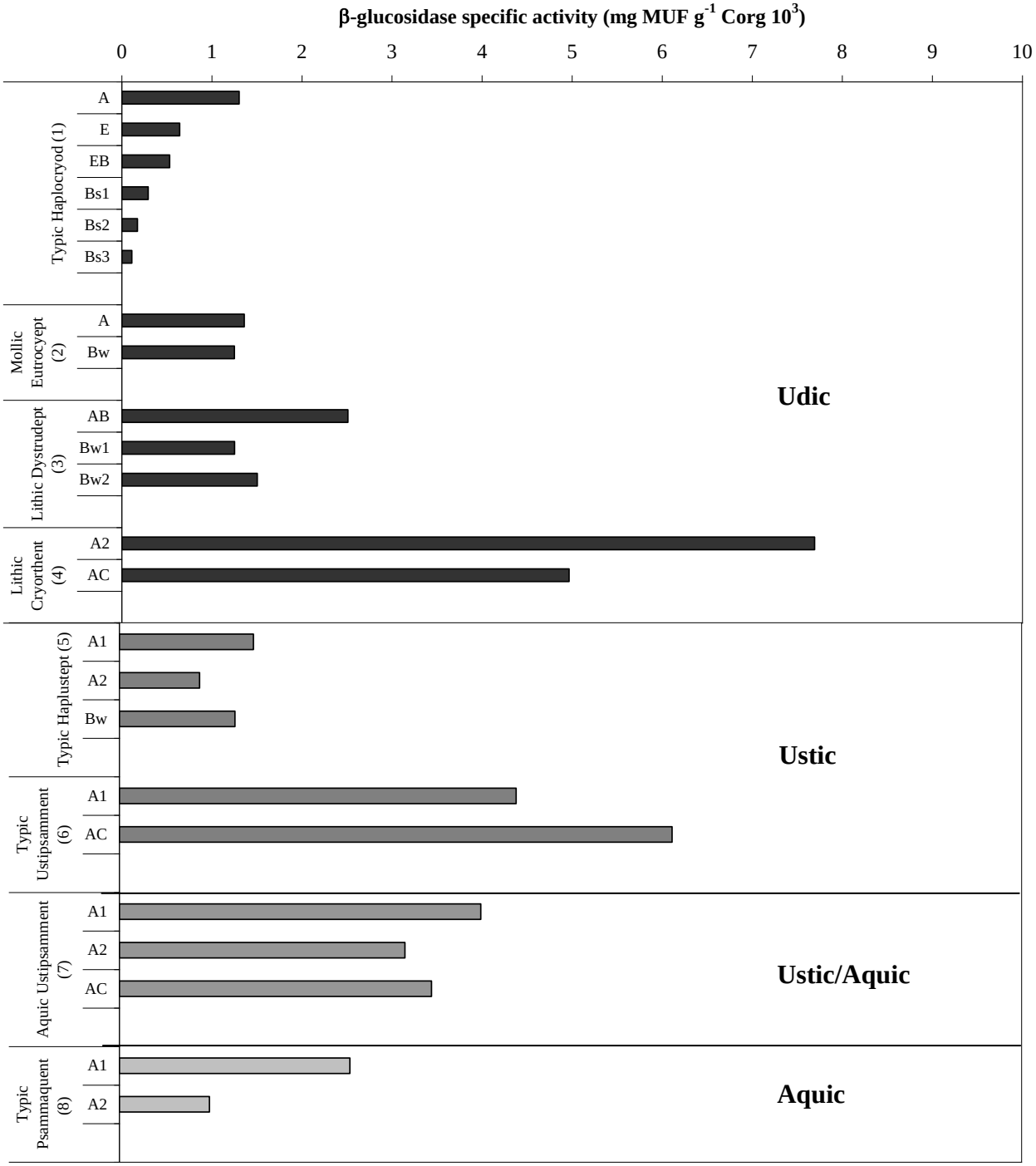


Figure 3: Specific activity (per unit of organic carbon) of β-glucosidase along soil profiles under Udic, Ustic and Aquic soil moisture regimes soil moisture regimes.

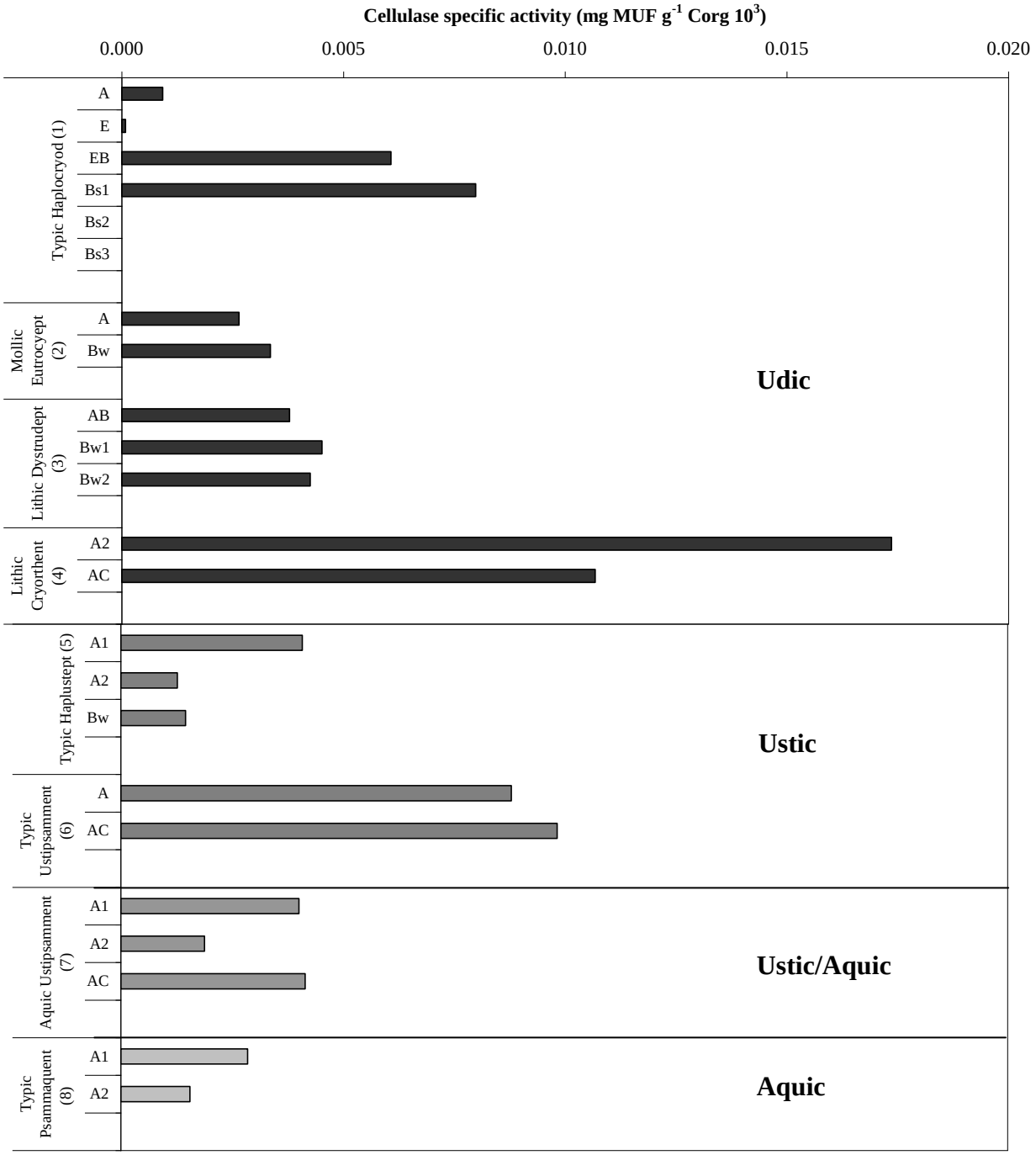


Figure 4: Specific activity (per unit of organic carbon) of cellulase along soil profiles under Udic Ustic and Aquic soil moisture regimes

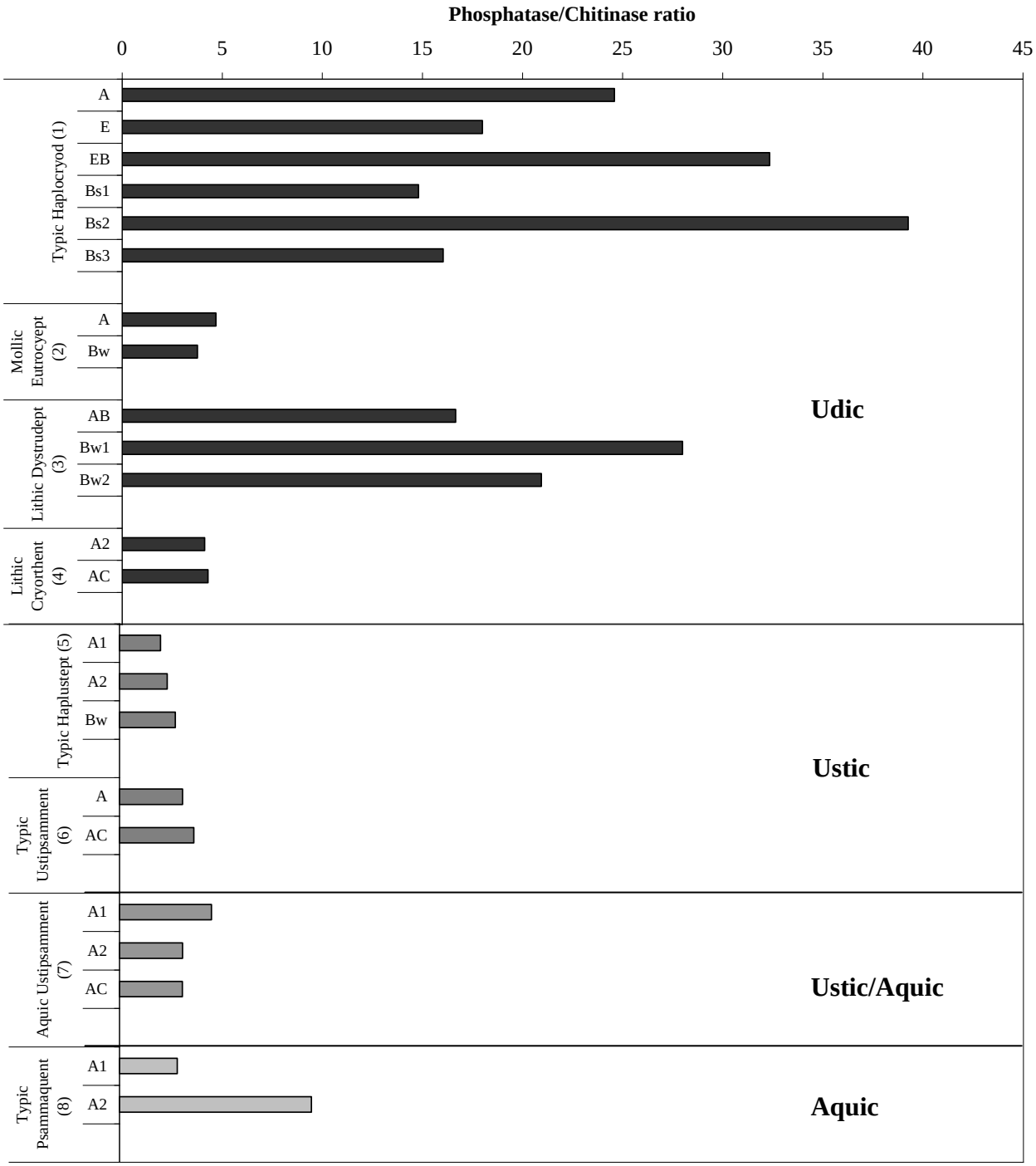


Figure 5: Soil phosphatase: chitinase ratio along soil profiles under Udic, Ustic and Aquic moisture regimes.

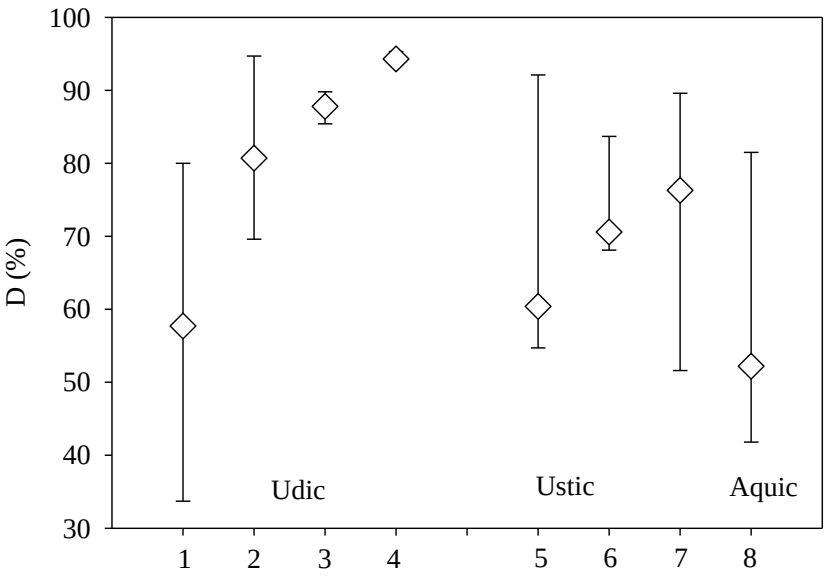


Figure 6: Gini diversity coefficient measured in eight soil profiles under Udic, Ustic and Aquic regimes. Diamonds represent the value calculated for each profile, bars represent maximum and minimum values within each profile.

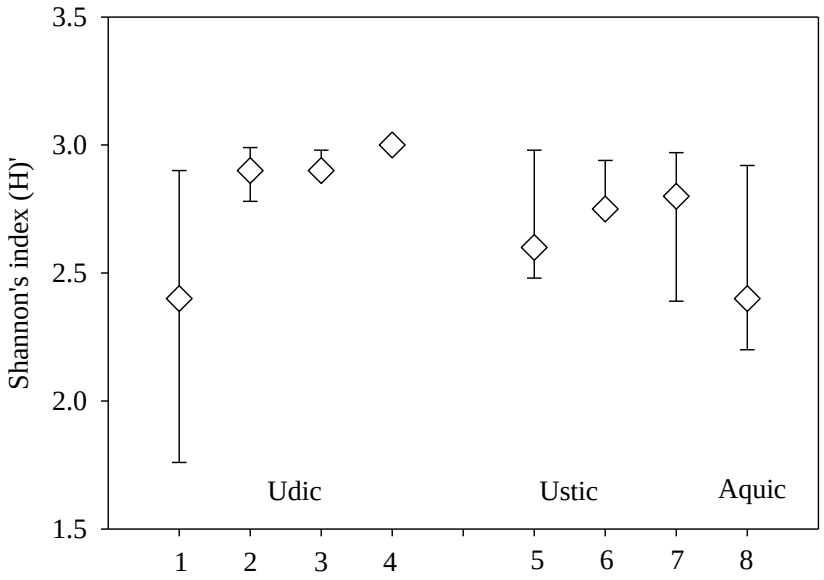


Figure 7: Shannon diversity index measured in eight soil profiles under Udic, Ustic and Aquic regimes. Diamonds represent the value calculated for each profile, bars represent maximum and minimum values within each profile.

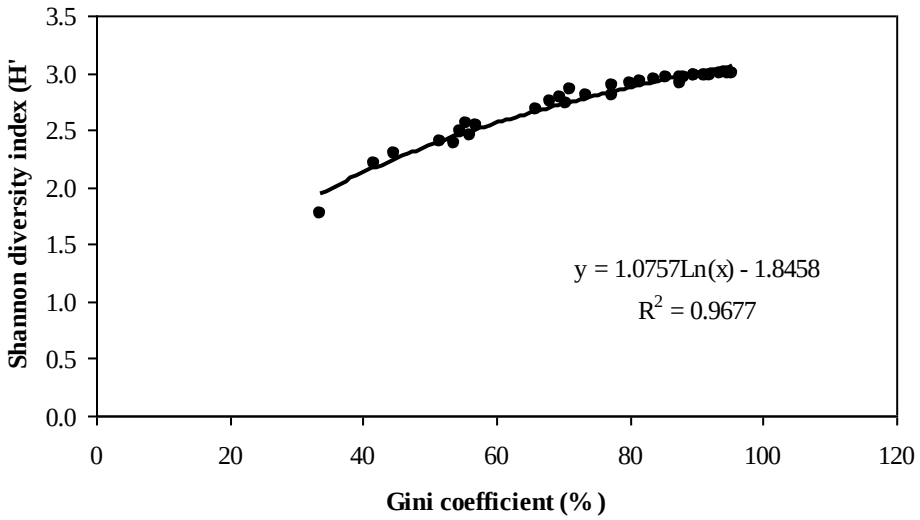


Figure 8: Gini coefficient vs. Shannon's diversity index.

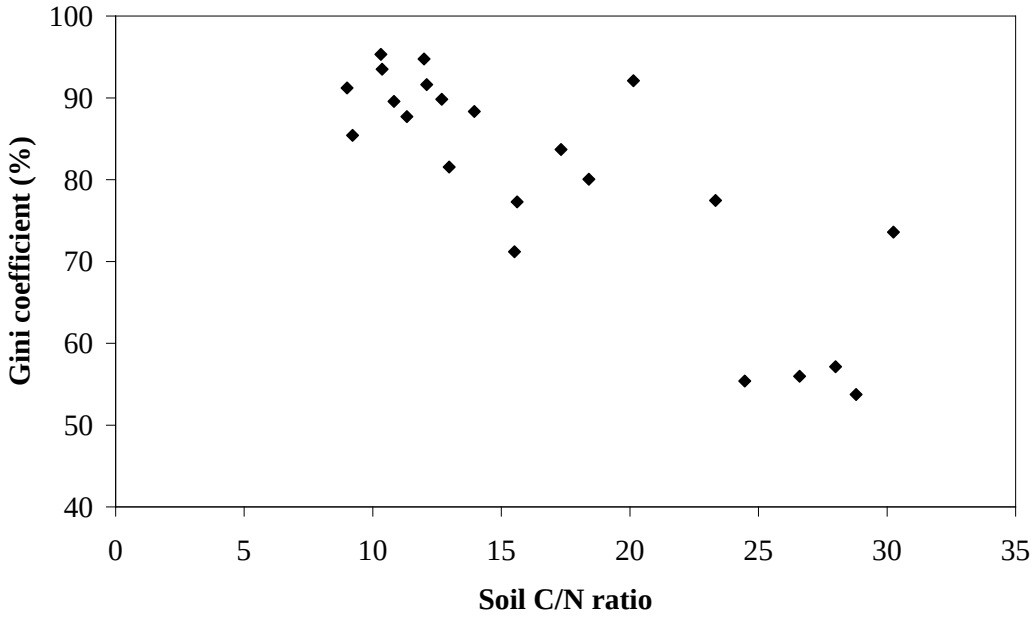


Figure 9: Microbial functional diversity (Gini coefficient) with respect to soil C/N ratio