SOIL MICROBIAL ACTIVITY IN HYDROMORPHIC-SUBAQUEOUS ECOSYSTEMS: PROCESSES AND FUNCTIONAL BIODIVERSITY

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Abstract

The hydromorphic and subaqueous soils have largely been overlooked on their pedogenic concepts or in soil C accounting studies considering their phisico-chemical properties. Conversely, little attention has been paid to the microbial activity playing a key role in regulating the biogeochemical cycle of elements. The aim of the study was to evaluate biological properties such as enzyme activities and the functional diversity of soil microbial population as bio- indicators, sensitive to processes affected by the water shallow. Eight soil profiles were opened along two transects: 1) a-a' North and 2) b-b' South, in a dune ecosystem of the Adriatic coast, Ravenna (Italy). The soil chemical and biochemical properties were determined. In particular, soil enzyme activities and soil induced respiration were measured using the microplates technique in order to assess the microbial functional diversity. The soil biochemical properties such as the potential enzyme activities and microbial induced respiration, as well as microbial functional diversity were sensitive indicators of soil processes in hydromorphic and subaqueous environment.. A general reduction of hydrolytic enzyme activities was observed in subaqueous soil with respect to hydromorphic one. Moreover, the endopedon of subaqueous soils showed a lower microbial functional diversity than hydromorphic one. In this study the ratio of enzyme activities involved in C to S cycles (SEIC/Aryl) as well as the C:S ratio showed a marked reduction in the subaqueous with respect to hydromorphic soils. In conclusion the C and S biogeochemical cycles of hydromorphic and subaqueous soils, in a coastal area may depend on freshwater and saltwater interface equilibrium.
Keywords: *Sulfiwassent, Psammowassent, Psammaquent, soil microbial activity.*

Introduction

The Adriatic coast area in the province of Ravenna is subject to subsidence (Teatini et al., 2005), the water tables are below sea level and saltwater has replaced freshwater in the aquifer (Antonellini et al., 2008). Moreover, the saltwater intrusion in the dune system of the Ravenna coast has produced hydromorphic soil conditions. Water-saturated zones are typical for hydromorphic soils, which are characterized by groundwater close to the soil surface (20–100 cm) (Buscaroli et

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al., 2009). There is limited information regarding the spatial distribution of soil properties or sediment in subaqueous soil science of shallow-water habitats. This limitation in part stems from the lack of an adequate model or system to classify and delineate subaqueous soil types (Demas et al., 1996). Moreover, subaqueous soil can present difficult situations to be studied due to thermodynamic limits: (1) turbulent exchange between the land surface and the atmosphere, (2) mechanical weathering from periodic heating and cooling of the ground, and (3) the transport of sediments by river flow (Kleidon et al., 2012). However, both hydromorphic and subaqueous soils are extremely complex ecosystems being characterized by pedogenic factors not included in Jenny's equation (Demas, 2001).
In these soils, the long periods of water saturation impose the condition of anaerobiosis for microbial populations. They are characterized by a wide biological diversity containing, in some cases, more species than in terrestrial and aquatic habitats (Liesack et al., 2000). The origin of the microbial biodiversity is due to the soil heterogeneity at the micro and macro-scale and to the considerable microbial competition for sources of energy and nutrition. The aim of the study as to evaluate the enzyme activities and the functional diversity of soil microbial population as biological indicators, sensitive to processes affected by the water shallow.

Material and methods

In a dune ecosystem of the Adriatic coast in Ravenna province eight soil profiles were opened along two transects: 1) a-a' North and 2) b-b' South. In each transect two hydromorphic soils and two submerged soils were identified (Fig.1). The hydromorphic soils were classified as Sodic and Typic Psammaquent while the subaqueous soils were Sulfic and Typic Psammowassent or Fluventic Sulfiwassent. The soils were sampled and frozen (-20 °C) for few days, then before biochemical analysis, soils were left for three days at room temperature to 60% of the water holding capacity. The chemical properties of soils were determined using the official methods, while the soil potential enzyme activity was determined using the microplates technique and the specific fluorogenic substrates of the following enzymes: β-cellobiohydrolasi (EC 3.2.1.91), N-acetyl-α-glucosaminidase (EC 3.2.1.30), α-glucosidase (EC 3.2.1.21), α-glucosidase (EC 3.2.1.20), phosphomonoesterase (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) (Marx et at., 2001; Vepsäläinen et al., 2001). The enzyme activity was expressed as specific activity (per unit of organic carbon) and as synthetic index given by the sum of all enzyme activities (SEI) or specifically for those of enzymes involved in the carbon cycle (SEIc). Finally, the ratio of the SEIc to arylsulfatase ratio was calculated to be related to the C: S ratio. Moreover, the respiration induced by the addition of 15 organic substrates (SIR) was determined by the Microresp method (Campbell et al. 2003) by placing the soil in optimum conditions of moisture and temperature. The Shannon diversity index (H') was calculated using both enzyme activities and SIR according to the following formula:

$$H' = \Sigma pi \ln pi \qquad [1]$$

where pi is the ratio of the activity of a particular enzyme to the sum of activities of all enzymes (Bending et al., 2002) or the sum of the respiratory activity induced by various substrates added to soil. The chemical and biochemical properties of the epipedon and endopedon were used for the multivariate discriminant analysis (Differential Function Analysis, DFA) in order to verify the homogeneity of the soil groups.

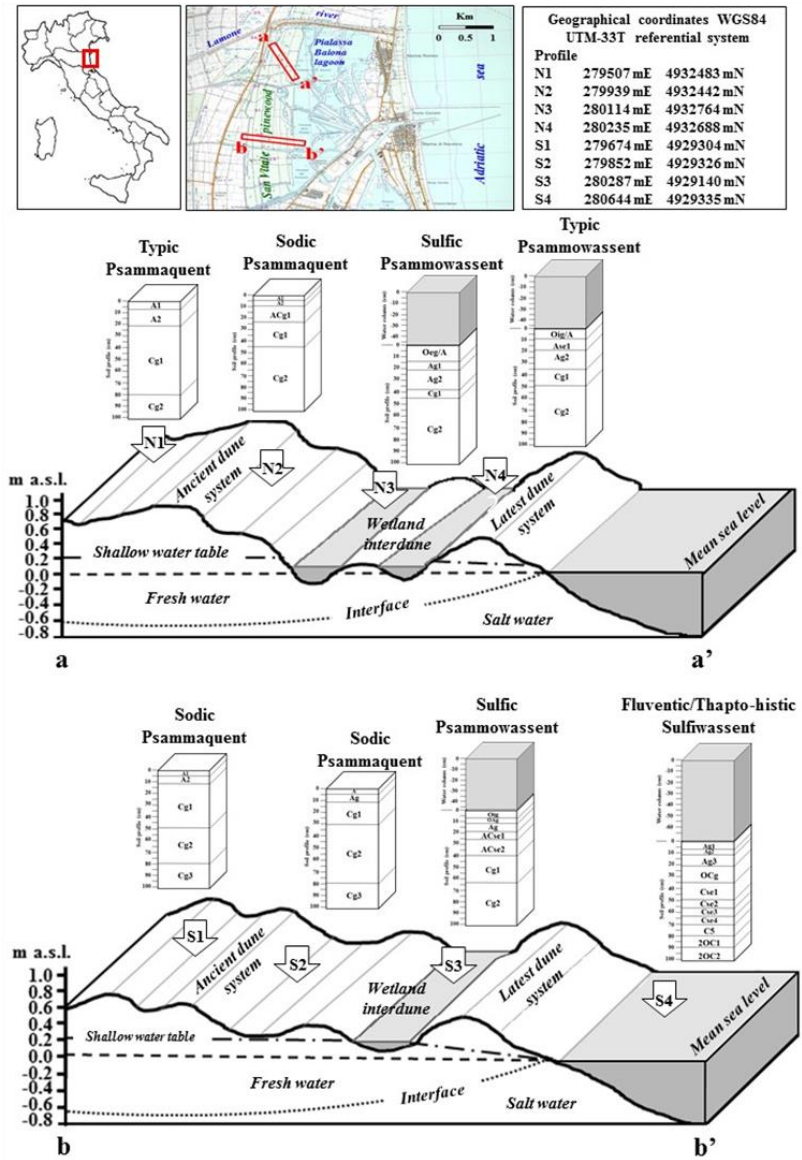

Figure 1 *Site description of north (a-a') and south (b-b') transects.*

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Results and discussion

The groundwater in the coastal area of Ravenna (San Vitale Pinewood) produced a wide diversification of the physico-chemical and biochemical properties of soil (table 1 and 2, respectively). In particular, the epipedon of subaqueous soils showed carbonate content similar to endopedon; while in the hydromorphic soils a general reduction of carbonate was observed particularly in the epipedon (Table 1). Similar trend was observed for electrical conductivity and soil pH which are depending on each other. The total organic carbon content (Corg) in the subaqueous soils was lower in the epipedon and higher in the endopedon with respect to the hydromorphic ones (Table 1). There are limits to the suggestion that slow decay in water-saturated zones is explained by the low free-energy yield of anaerobic respiration. However, it is known little about the factors that regulate fermentative bacteria, enzyme activity, substrate feedbacks and microbial community interactions — all of which affect organic matter accumulation (Kirwan and Megonigal, 2013).

Finally, the C:S ratio was significantly lower in the subaqueous than in the hydromorphic soils (Table 2). It is known that the delivery of salts and sulphates to brackish and freshwater coastal wetlands through sea-level rise may destabilize soil organic matter pools (Kirwan and Megonigal, 2013). Organic accretion rates tend to be highest in freshwater tidal wetlands, and studies report accelerated decomposition rates with saltwater intrusion, but as discussed by Kirwan and Megonigal (2013) these results are equivocal and we lack the mechanistic insight to explain such responses. In this study similar behavior of C:S ratio was observed for the ratio between the enzyme activities involved in C and S cycle (SEIc/Aryl ratio). These results suggest that both, C and S biogeochemical cycles in the coastal wetland may depend on soil hydrology in particular on the freshwater and saltwater interface equilibrium. In addition, the specific enzyme activity (per unit of organic carbon) of arylsulphatase, butyrate esterase and phosphomonoesterase was usually lower in the subaqueous soils than in the hydromorphic ones (table 2). Therefore, as reported in a previous study, the water saturation conditions mainly change the efficiency of organic substrate hydrolysis (Marinari et al., 2012).

The soil microbial respiration induced by the addition of substrates was highest in the epipedon of subaqueous than the hydromorphic soils (Fig. 2A). In contrast, the whole soil enzyme activity, expressed by the Synthetic Enzymatic Index (SEI), was lower in subaqueous than hydromorphic soils (Figure 2B). Moreover, the functional diversity of the microbial population, measured in terms of respiration induced by the addition of substrates (H'SIR) or by enzyme activities (H'Enz) was significantly higher in endopedon of hydromorphic than of subaqueous (Fig. 3A and 3B). Conversely, the microbial functional diversity, calculated on enzyme activities basis (H'Enz), was higher in epipedon of subaqueous than in hydromorphic soils (Fig. 3B). Finally, the discriminant function analysis (Fig. 4) showed a clear separation of the hydromorphic epipedon and endopedon (Sodic and Typic Psammaquent).

Table 1. *Soil chemical properties of epipedon and endopedon in the north (a-a')and south (b-b') transects. Data are average values ± standard error (n=3)*

	$CaCO_3$ g kg^{-1}		pH		EC mS cm^{-1}		Corg g kg^{-1}		TN g kg^{-1}		CEC $Cmol^+$ kg^{-1}		Cextr mg kg^{-1}		S mg kg^{-1}	
	EPI	**ENDO**	**EPI**	**ENDO**	**EPI**	**ENDO**	**EPI**	**ENDO**	**EPI**	**ENDO**	**EPI**	**ENDO**	**EPI**	**ENDO**	**EPI**	**ENDO**
N1	**23** ±12	**118** ±1	**7.7** ±0.4	**8.5** ±0.0	**4.1** ±0.0	**1.2** ±0.1	**72.0** ±46.1	**4.2** ±1.2	**4.9** ±2.7	**0.3** ±0.2	**4** ±4	**48** ±8	**12** ±1	**44** ±8	**1422** ±824	**81** ±13
N2	**17** ±6	**127** ±13	**8.3** ±0.2	**8.6** ±0.0	**3.0** ±0.0	**3.1** ±0.4	**35.3** ±14.0	**2.7** ±0.6	**3.1** ±1.0	**0.3** ±0.1	**3** ±1	**63** ±10	**283** ±10	**80** ±19	**730** ±176	**245** ±30
N3	**170** ±5	**227** ±24	**8.3** ±0.1	**8.9** ±0.1	**6.6** ±1.4	**7.1** ±0.2	**9.7** ±1.6	**11.9** ±2.4	**1.0** ±0.0	**1.4** ±0.2	**7** ±6	**30** ±2	**145** ±64	**172** ±14	**5526** ±2925	**5407** ±1657
N4	**127** ±5	**150** ±13	**7.8** ±0.0	**8.3** ±0.1	**4.4** ±0.6	**2.4** ±0.1	**22.3** ±2,2	**6.2** ±3.8	**2.3** ±0.6	**0.8** ±0.3	**4** ±3	**10** ±5	**195** ±61	**66** ±22	**3570** ±250	**778** ±425
S1	**20** ±6	**73** ±12	**7.3** ±0.3	**8.3** ±0.1	**1.3** ±0.6	**0.2** ±0.0	**53.9** ±17,3	**1.5** ±0.3	**3.9** ±1.6	**0.4** ±0.3	**1** ±1	**26** ±7	**1490** ±859	**39** ±28	**1520** ±549	**109** ±7
S2	**9** ±0	**61** ±27	**7.0** ±0.3	**7.7** ±0.4	**5.3** ±0.1	**3.4** ±0.9	**64.2** ±12.5	**11.6** ±8.7	**4.7** ±1.0	**0.9** ±0.7	**5** ±5	**39** ±6	**295** ±45	**69** ±31	**1516** ±49	**562** ±159
S3	**271** ±41	**122** ±1	**8.1** ±0.0	**8.6** ±0.0	**16.4** ±2.7	**6.8** ±0.0	**34.9** ±10,0	**3.4** ±0.0	**3.8** ±1.3	**0.2** ±0.0	**16** ±6	**7** ±0	**469** ±266	**134** ±5	**5759** ±1565	**1545** ±9
S4	**143** ±31	**115** ±9	**8.8** ±0.2	**8.9** ±0.3	**5.0** ±5.0	**10.8** ±1.6	**13.4** ±10.2	**2.6** ±0.1	**1.3** ±1.1	**0.1** ±0.0	**5** ±0	**5** ±0	**485** ±309	**152** ±56	**2899** ±1148	**1562** ±85

EC = Electrical conduttivity; **Corg** = total organic carbon **–** **TN** = total nitrogen; **CEC** = exchangeable cations capacity; **Cextr** = extractable carbon; **S** = sulfur

Table 2. *Soil biochemical properties of epipedon (EPI) and endopedon (ENDO) in the north (a-a') and south (b-b') transects. Data are average value ± standard error (n=3).*

	SEI		C:S		SEIc/Aryl		Aryl/Corg		But/Corg		Pho/Corg	
	EPI	**ENDO**	**EPI**	**ENDO**	**EPI**	**ENDO**	**EPI**	**ENDO**	**EPI**	**ENDO**	**EPI**	**ENDO**
N1	**2550** ±505	**670** ±218	**48** ±5	**50** ±11	**7.7** ±2.8	**13.8** ±2.0	**6** ±1	**12** ±2	**75** ±27	**1197** ±512	**47** ±21	**133** ±43
N2	**1492** ±390	**212** ±20	**46** ±8	**12** ±4.1	**1.3** ±0.2	**3.9** ±1.3	**35** ±1	**25** ±4	**218** ±54	**998** ±214	**66** ±13	**123** ±19
N3	**28** ±7	**0** ±0	**2** ±1	**2** ±0	**1.3** ±0.0	**0.08** ±0.04	**2** ±1	**0** ±0	**58** ±7	**53** ±8	**20** ±4	**14** ±2
N4	**159** ±5	**25** ±25	**6** ±0	**9** ±2	**0.6** ±0.0	**0.3** ±0.1	**11** ±1	**3** ±2	**113** ±5	**81** ±26	**21** ±1	**47** ±26
S1	**1059** ±624	**41** ±3	**36** ±2	**14** ±2	**4.3** ±3.5	**1.1** ±0.1	**9** ±6	**24** ±5	**102** ±23	**1523** ±476	**22** ±7	**35** ±8
S2	**688** ±391	**251** ±76	**47** ±7	**16** ±9	**1.3** ±0.6	**2.7** ±1.0	**8** ±1	**24** ±14	**61** ±0	**406** ±172	**23** ±11	**51** ±23
S3	**121** ±110	**0** ±0	**6** ±0	**2** ±0	**0.4** ±0.2	**0.0** ±0.0	**4** ±3	**1** ±0	**49** ±28	**46** ±0	**23** ±21	**20** ±0
S4	**30** ±18	**4** ±4	**4** ±2	**2** ±0	**0.4** ±0.1	**0,5** ±0,5	**7** ±1	**3** ±1	**71** ±28	**99** ±4	**26** ±7	**36** ±4

SEI = Synthetic Enzymatic Index; **C:S** = carbon to sulfur ratio; **SEIC/Aryl** = ratio of Synthetic Index of enzyme involved in C cycle to arylsulfatase enzyme activity; **Aryl/Corg** = specific arylsulfatase activity; **But/Org** = specific activity of butirate esterase; **Pho/Corg** = specific activity of acid phosphomonoesterase.

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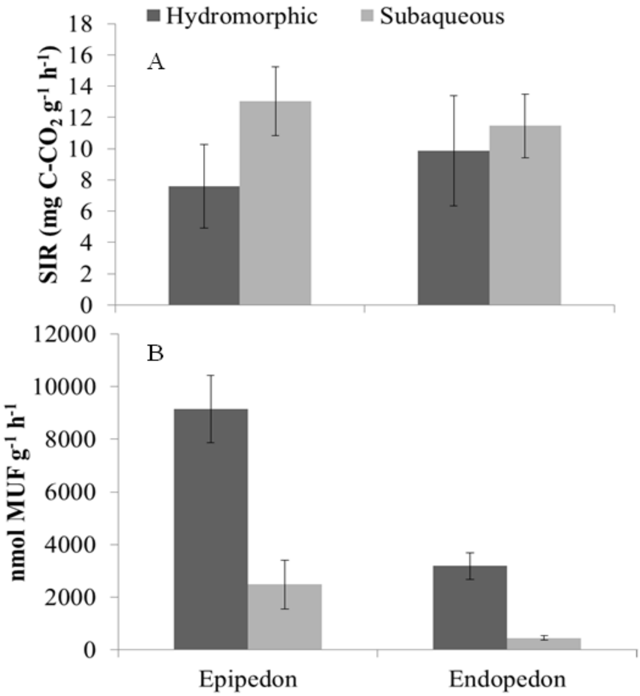


Figure 2
Substrates induced respiration (A) and synthetic enzimatic index (SEI) (B) in the epipedon and endopedon of hydromorphic and subaqueous soils. Bars are standard errors (n=4)

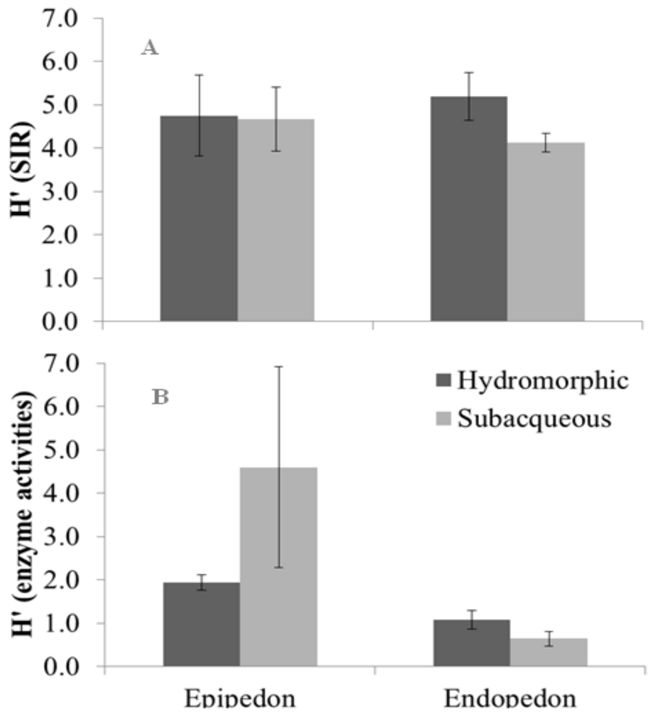
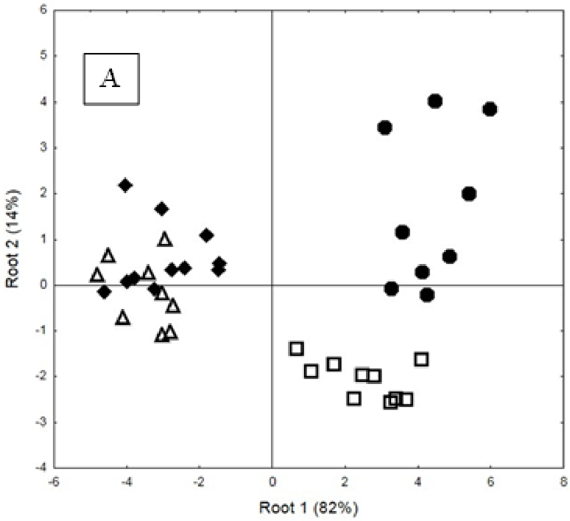
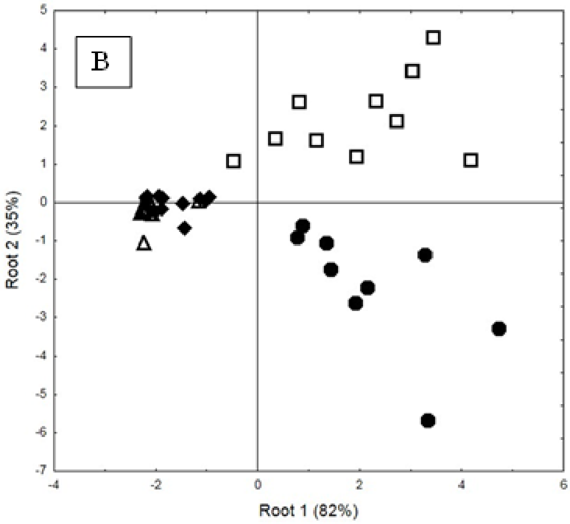


Figure 3
Shannon's diversity Index (H') in epipedon and endopedon of hydromorphic and subaqueous soils. H' is calculated using the induced respiration activities (A) and enzyme activities (B). Bars are standard errors (n=4)

This separation was not significant in subacqueous soils (Sulfic and Typic Psammowassent, Fluventic Sulfiwassent). The chemical variables that were significantly correlated with one of the plot axes are shown in the table next to figure 4A.



	Root 1	Root 2
SEIc	0.66 (***)	-0.62 (***)
C/S	0.86 (***)	-0.34 (*)
SEIC/Sulfatasi	0.60 (***)	ns
Aryl/TOC	0.66 (***)	ns
But/TOC	0.53 (***)	0.73 (***)
Fosf/TOC	0.50 (***)	0.49 (***)



	Root 1	Root 2
$CaCO_3$	-0.77 (***)	ns
pH	-0.49 (**)	-0.50 (**)
EC	-0.58 (***)	ns
TOC	0.40 (*)	0.82 (***)
TN	ns	0.83 (***)
CEC	0.67 (***)	ns
WEOC	ns	0.49 (**)
P	ns	ns
S	-0.57 (***)	0.39 (*)

Figure 4. *Discriminant function analysis of soil physico-chemical (A) and biochemical properties (B) in the epipedon (EPI) and endopedon (ENDO) of hydromorphic (PIN) and subaqueous soils (WAS). In tables are showed the correlation coefficients between axes (root 1 e root2) and the main soil properties* *** $p<0.001$*;* ** $p<0.01$*;* * $p<0.05$*, ns not significant.*

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In particular, those significantly correlated with both axes (Root1 and Root2) also describing the separation of the three groups (hydromorphic epipedon, hydromorphic endopedon and subaqueous soils) were: pH, organic carbon and sulfur. The biochemical variables correlated with the plot axes of the DFA are reported in the table next to figure 4B.

Conclusion

The soil biochemical properties such as the potential enzyme activities and microbial induced respiration, as well as microbial functional diversity were sensitive indicators of soil processes in hydromorphic and subaqueous environments. However, it is important to emphasize that the adopted methods placed the soil microbial population under optimal conditions of humidity and temperature, therefore microbial induced respiration measured as potential activity, certainly differs from the real one. Finally, in hydromorphic and subaqueous soils of a coastal area the C and S biogeochemical cycles, may depend on freshwater and saltwater interface equilibrium. In this study the ratio of enzyme activities involved in C to S cycles (SEIC/Aryl) as well as the C:S ratio showed a marked reduction in the subaqueous with respect to hydromorphic soils.

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