Disentangling drivers of soil microbial potential enzyme activity across rain regimes: An approach based on the functional trait framework

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Abstract

The functional trait framework provides a powerful corpus of integrated concepts and theories to assess how environmental factors influence ecosystem functioning through community assembly. While common in plant ecology, this approach is under-used in microbial ecology. After an introduction of this framework in the context of microbial ecology and enzymology, we propose an approach 1) to elucidate new links between soil microbial community composition and microbial traits; and 2) to disentangle mechanisms

underlying “total” potential enzyme activity in soil (sum of 7 hydrolase potential activities). We address these objectives using a terrestrial grassland ecosystem model experiment with intact soil monoliths from three European countries (Switzerland, France and Portugal) and two management types (Conventional-intensive and Ecological-intensive), subjected to 4 rain regimes (Dry, Wet, Intermittent and Normal) under controlled conditions in a common climate chamber. We found tight associations between proxies of microbial ecoenzymatic community-weighted mean traits (enzymatic stoichiometry and biomass-specific activity) and community composition, bringing new information on resource acquisition strategy associated with fungi, Gram positive and Gram negative bacteria. We demonstrate that microbial biomass explained most of the total enzyme activity before altered rain regimes, whereas adjustments in biomass-specific activity (enzyme activity per unit of microbial biomass) explained most variation under altered rain regime scenarios. Furthermore, structural equation models revealed that the variation of community composition was the main driver of the variation in biomass-specific enzyme activity prior to rain perturbation, whereas physiological acclimation or evolutionary adaptation became an important driver only under altered rain regimes. This study presents a promising trait-based approach to investigate soil microbial community response to environmental changes and potential consequences for ecosystem functioning. We argue that the functional trait framework should be further implemented in microbial ecology to guide experimental and analytical design.

Keywords: bacteria; climate change; enzymatic stoichiometry; fungi; PLFA; structural equation model

1 Introduction

Theoretical framework based on the functional trait concept provides a wide corpus of integrated concepts and theories at different levels to address organisms’ adaptation, community assembly and ecosystem functioning (Lavorel and Garnier 2002, Diaz et al. 2007,

Violle et al. 2007). Functional traits are defined as any physiological, morphological, phenological or genomic feature, measured at the individual level, and affecting the fitness or function of an organism (Violle et al. 2007, Krause et al. 2014). Integrated at the community level, functional traits underlie the community functional composition often characterized by the community weighted mean (CWM) trait and the trait diversity (Diaz et al. 2007, Violle et al. 2007). These emergent properties at the community level are controlled by mechanisms at the individual (physiological acclimation), population (adaptation) and community (species turnover) scales (Violle et al. 2007) and they are considered as major drivers of ecosystem functioning (Grime 1998, Petchey and Gaston 2006, Diaz et al. 2007). Hence, the linkages between response traits (controlling organism response and adaptation to environmental changes) and effect traits (controlling organism effect on ecosystem functioning), and their integration at the community level, provide a mechanistic basis to understand community assembly and cascading effects on ecosystem functioning (Lavorel and Garnier 2002, Litchman et al. 2015) (Figure 1). Such multiscale integrated framework is essential to correctly interpret complex ecological data. However, functional trait framework has hardly been used in microbial ecology and further studies are required to develop how it can be successfully used for soil microbial communities (Piton et al. 2019).

Plant and microbial traits associated with resource acquisition are both response and effect traits (Lavorel and Garnier 2002, Litchman et al. 2015), making them promising candidates for inclusion into mechanistic models of ecosystem functioning (Allison 2012). Extracellular decomposition of organic matter and subsequent assimilation of its depolymerized compounds are central in the resource acquisition strategies of heterotrophic soil microbes (Sinsabaugh and Follstad Shah 2012). Traits associated with extracellular enzyme production (ecoenzymatic traits) and the uptake of nutrients are probably key in microbial physiological and evolutionary trade-offs (Malik et al. 2019a) (Figure 1). Indeed,

the production of extracellular enzymes bears high energy and nitrogen (N) costs for microbes (Frankena et al. 1988, Allison et al. 2010), at the expense of the investment in other metabolic pathways such as growth, cellular maintenance and stress tolerance (Malik et al. 2019a, Ramin and Allison 2019). Hence, it has been proposed that oligotrophic microbial species in resource-poor environments invest more in extracellular enzymes to cope with low resource availability compared to copiotrophic species with a growth oriented strategy dominating in resource-rich environments (Fontaine et al. 2003, Fierer et al. 2007). Trade-offs also exist between the production of different enzymatic classes since enzymes should match with substrate availability, while satisfying the nutritional need of the microbial cell (Figure 1). Biomass stoichiometry is relatively constrained in heterotrophic microbes (Fanin et al. 2013, Zechmeister-Boltenstern et al. 2015), with high biomass C:N and N:P ratios reported in fungi relative to bacteria and in oligotrophic microbes relative to copiotrophic ones (Fierer et al. 2007, Strickland and Rousk 2010, Litchman et al. 2015). To match these stoichiometric constraints, the resource allocation model (Sinsabaugh et al. 1993) predicts microbes to optimize the enzyme production for C, N and P acquisition toward the most limiting element to maximize their fitness. Following this theory, enzymatic stoichiometry, that is the relative investment by microbes for C, N or P acquisition enzymes (Sinsabaugh et al. 2009), can be considered as a proxy of the resource acquisition strategy that should be adapted to the nutritional constraint on microbial communities. Oligotrophic microbial communities dominating nutrient poor soils are expected to direct their resource acquisition strategy toward nutrient (N and P) acquisition, whereas copiotrophic microbes should display an opposed stategy (C acquisition) in nutrient rich soils. However, recent empirical results (Rosinger et al. 2019) challenge this theory and suggest that the nutritional constraint is not the only factor controlling enzymatic stoichiometry.

Measuring functional traits, requires measurement at the individual level, which is very challenging for microbes (Martiny et al. 2015). However, Piton et al (2019) demonstrated that measuring biomass-specific potential enzyme activity (potential activity per unit of microbial biomass) and enzyme stoichiometry give a direct approximation of community-weighted mean (CWM) traits representative of the dominant strategy in the microbial community. Using such indicators, Malik et al. (2019c) and Piton et al. (2019) observed decreases in mass-specific extracellular potential enzyme activity along soil resource gradients. Their results indicate that oligotrophic microbes invest more in the production of extracellular enzymes as compared to copiotrophic ones, with these extracellular enzymes especially oriented toward nutrient acquisition (especially P in Piton et al. 2019), consistent with theoretical expectation (Sinsabaugh et al. 1993, Fontaine et al. 2003, Malik et al. 2019a). Consequently, ecoenzymatic CWM traits (biomass-specific activity and enzymatic stoichiometry) are promising candidates to understand how the response of soil microbial communities to environmental changes and its cascading effect on ecosystem functioning.

Extracellular enzyme activity in soils is central for ecosystem functioning as it controls decomposition and mineralization of soil organic matter (Schimel and Bennett 2004, Bengtson and Bengtsson 2007). Firstly, this activity depends on the enzyme concentrations in soil and their catalytic properties (e.g. the catalytic turnover rate representing the number of substrates molecules converted to product per enzyme per unit of time). These two parameters drive the extracellular enzymatic potential activity (V_{max}), commonly measured under laboratory condition without constraint of substrates concentration and diffusion, often at a single temperature and a single pH (Wallenstein and Weintraub 2008). The realized *in situ* activity is more difficult to assess but can be modelled, based on this potential activity and the

121 environmental conditions (Wallenstein and Weintraub 2008, Steinweg et al. 2012, Allison and
122 Goulden 2017).
123 Total extracellular enzyme potential activity is controlled by microbial mechanisms
124 scaling from individual to community level (Sinsabaugh 2005, Burns et al. 2013).
125 Extracellular enzymes are broadly produced among soil microbes (Allison et al. 2007a,
126 Vranova et al. 2013), so that soil enzyme potential activity is assumed to be firstly controlled
127 by the microbial biomass (Kivlin et al. 2013). However, a decoupling between microbial
128 biomass and enzyme potential activity can be induced by enzyme stabilization on inorganic
129 surfaces and organic colloids and persistence after the death of their producers (Nannipieri et
130 al. 2018), or through differences among microbes in extracellular enzyme production per unit
131 of microbial biomass (Allison et al. 2007b, Burns et al. 2013, Kivlin et al. 2013, Steinweg et
132 al. 2013). Variations in the biomass-specific enzyme activity measured at the community
133 level (the CWM trait) can emerge both from changes in community composition (Li et al.
134 2019), as well as from the community members' physiological acclimation to environmental
135 changes (Schimel et al. 2007) or evolutionary adaptation (Allison et al. 2018).
136 Today with novel molecular and culturing techniques, there is evidence for a large
137 variation of enzyme production across microbial taxa (Lladó et al. 2016, Manoharan et al.
138 2017, Žifcáková et al. 2017). For instance, at broad taxonomic scale, a more important
139 production of enzymes for fungi is expected compared to bacteria, explaining their succession
140 during litter decomposition (Sinsabaugh 2005). Nevertheless, the importance of bacterial
141 enzyme activity in soils has been shown (Manoharan et al. 2017, López-Mondéjar et al.
142 2019). Several studies also indicate a variation in enzymatic investment within bacterial and
143 fungal groups (Lladó et al. 2016, Pierre-Emmanuel et al. 2016). Gram positive and Gram
144 negative bacteria are considered as oligotrophic and copiotrophic respectively (Fierer et al.
145 2007, Fanin et al. 2018). Gram positive bacteria use more recalcitrant carbon (C) compounds

146 and produce more enzymes to extract energy and nutrients from organic matter.
147 Comparatively, Gram negative bacteria use labile C compounds and produce less enzymes
148 (Fanin et al. 2018, Naylor and Coleman-Derr 2018). Together these studies suggest the
149 potential important contribution of the microbial community composition to ecosystem
150 functioning (Graham et al. 2016) through its links with CWM biomass-specific activity.
151 Finally, experimental results also provide some supports for a physiological acclimation of
152 microbial community members or for an evolutionary adaptation of their population in
153 response to environmental changes (Allison et al. 2014, 2018, Lashermes et al. 2016),
154 affecting enzymes production independently of community composition or microbial biomass
155 changes, potentially also contributing to variations of CWM biomass-specific activity.
156 To sum up, the control of potential extracellular enzyme activity in soil relies on four
157 parameters: microbial biomass and three parameters potentially influencing biomass-specific
158 activity: community composition, community members' acclimation/adaptation and enzyme
159 abiotic stabilization (reduction of enzymes turnover by abiotic factors). To assess the relative
160 importance of these four parameters, their physical control in a manipulated experiment
161 would be very difficult and implicate a highly artificial environment. Structural equation
162 model (SEM) framework can be used as an alternative to statistically assess the role of
163 different mechanisms underlying observed responses in experimental or observational studies
164 where factors affecting the processes under investigation cannot be physically controlled
165 (Shipley 2016).
166 Based on experimental data assessing ecosystem functioning across different management
167 (conventional intensive vs. ecological intensive) and countries (France, Switzerland and
168 Portugal) under 4 rain regime scenarios (Dry, Normal, Intermittent and Wet rain regimes,
169 during 263 days, followed by 89 days of recovery), we used ecoenzymatic CWM traits (Piton
170 et al. 2019): 1) to identify the links between traits and soil microbial community composition

171 along abiotic gradients; and 2) to disentangle mechanisms driving the potential enzyme
172 activity in soil.
173 We hypothesized:
174 1) A high biomass-specific activity (oligotrophic CWM trait), and a nutrient acquisition
175 strategy to be associated with fungi and/or Gram positive dominated communities, explaining
176 their dominance in low nutrients and low moisture conditions.
177 Then, we firstly assessed the relative importance of microbial biomass and biomass-
178 specific activity in the variation of the total enzyme activity (ecosystem level property).
179 Secondly, we used structural equation models to disentangle soil abiotic factors and microbial
180 community composition control of microbial biomass and biomass-specific activity.
181 2) We further hypothesized that:
182 Microbial biomass variation is the main driver of the total enzyme activity in soil, and
183 that altered rain regimes induce biomass-specific activity adjustment in response to resource
184 availability changes. Biomass-specific activity is mainly controlled by changes in microbial
185 community composition and to a lesser extent by community members'
186 acclimation/adaptation or enzyme abiotic stabilization.

187 ## 2 Material and methods

188 ### 2.1 Experimental design and setup

189 In this study, we used data from a continental scale experiment testing effects of 4 rain
190 regimes (normal, dry, wet and intermittent) on Terrestrial Model Ecosystems (TME) extracted
191 from grasslands representing dominant pedoclimatic and management conditions across
192 Europe (Table 1 and Lori et al. (2020) for details). One hundred and twenty TMEs (40 cm
193 depth x 16.5 cm diameter) encased in HDTPE tubes were collected. More precisely, four
194 different plots were sampled for each management (eco-intensive and conventional-intensive)

195 in each country (8 plots per county), with 5 TMEs extracted in each plot, using a
196 retroexcavator and a special stainless-steel extractor as described by Knacker et al. (2004).

197 After sampling, all TMEs were transported in a refrigerated truck to a single climate
198 chamber at the Laboratory of Soil Ecology and Ecotoxicology of Coimbra University. TMEs
199 were randomly placed inside special carts creating a temperature gradient between the lower
200 and the upper part as described by Ng et al. (2014). Air humidity was maintained at 60% and
201 temperature at 20˚C during the entire experiment and photoperiod was adjusted at 16h:8h
202 (light:dark).

203 During the first 81 days, artificial rainwater (Velthorst 1993) was added on each TME,
204 with the amount of water adjusted to obtain a soil moisture in the upper 20 cm layer (assessed
205 using Decagon moisture sensors) equivalent to 50%-60% of the maximum water holding
206 capacity (WHC_{max}) of the soil from each site where TMEs were collected. Those specific
207 values of soil moisture (50%-60% WHC_{max}) are considered as the "Normal" rain regime for
208 each country. After this acclimation period under "Normal" rain regime, the upper 10 cm of
209 soil were sampled on one TMEs (destructive sampling) to characterize initial state (T0).

210 After this acclimation period, 4 rain regimes were simulated during 263 days, with one rain
211 regime simulated on each of the four TME left from the 32 plots. Soil moisture was
212 maintained at 20-30%, 50%-60% and 70-80% of the WHCmax for Dry, Normal and Wet rain
213 regimes respectively. Intermittent rain regime was also simulated with 74 days under wet rain
214 regime followed by 125 days under dry regime and finally 64 days back to normal.

215 After this period (T1). One soil core of $98cm^3$ (5 cm diameter and 5 cm height) was
216 collected from each TME (non-destructive sampling) and pure sand encased into a small
217 plastic cylinder was used to fill the holes left after sampling. After this period of altered rain
218 regimes, all TMEs were set again to Normal rain regime for 89 days followed by a last
219 destructive sampling (T2) as described for T0 (upper 10 cm). At the 3 sampling times, soils

were sieved at 5 mm, plant roots were hand-sorted and samples were stored at 4°C or -20°C for further analyses.

2.2 Soil abiotic properties

Soil moisture was determined as the weight difference of a fresh soil sample after drying it for one week at 70°C, followed by 4 hr at 500°C to determine soil organic matter content (SOM) by loss on ignition. Soil pH was determined in a 1:6 (soil: 1M KCl) solution. Total soil N content was measured using an elemental analyzer (FlashEA 1112, Fisher Scientific, Waltham, Massachusetts, USA) on oven-dried subsamples ground to a fine powder (5 µm diameter) with a ball mill (MM301, Retsch GmbH, Haan, Germany).

2.3 Microbial community biomass and composition

Analysis of phospholipid fatty acids (PLFA) were used to characterize microbial biomass and community composition. Lipids were extracted from 3 g of soil according to Frostegård et al. (1993). Separation of the resulting fatty acid methyl esters was done on a Hewlett Packard 6890 gas chromatograph (column HP 5). PLFAs i15:0, a15:0, 15:0, i16:0, 16:1ω9, i17:0, a17:0, cy17:0, 18:1ω7, cy19:0 were chosen to represent bacterial biomass. PLFA 18:2ω6 was used as an indicator of fungal biomass (Frostegård and Bååth 1996). Gram positive biomass was indicated by i15:0, a15:0, i16:0, i17:0, a17:0 (O'leary and Wilkinson 1988), Gram negative bacteria biomass by PLFAs 18:1ω7, cy17:0, cy19:0 (Wilkinson 1988, Zelles 1997) and Actinobacteria biomass by 10Me17:0 and 10Me18:0 (Lechevalier and Moss 1977, Kroppenstedt 1985). The NLFA 16:1ω5 was used as an indicator for AMF biomass (Olsson et al. 1995). Microbial biomass-C was calculated based on the conversion factors: 363.6 nmol of bacterial-PLFA = 1 mg-C (Frostegård and Bååth 1996), 11.8 nmol of fungal-PLFA = 1 mg-C (Klamer and Bååth 2004) and 1.047 nmol of NLFA = 1 µg-C (Olsson et al. 1995).

243 The Fungal:Bacterial ratio and Gram+:Gram– ratio were calculated as Fungal biomass-C:
244 Bacterial biomass-C ratio (F:B here after), and Gram+ biomass-C: Gram– biomass-C ratio
245 (GP:GN hereafter) respectively. Relative abundances (% mol PLFA) of 27 identified PLFA
246 markers were used to characterize the overall microbial community composition.

247 2.4 Potential extracellular enzyme activities

248 Standard fluorimetric methods were used to measure potential extracellular enzymes
249 activity of seven enzymes degrading C-rich substrates (α-Glucosidase (AG), β-1,4-
250 Glucosidase (BG), β-D-Cellobiosidase (CB), and β-Xylosidase (XYL)), N-rich substrates (β-
251 1,4-N-acetylglucosaminidase (NAG) and leucine aminopeptidase(LAP)) and P-rich substrates
252 (phosphomonoesterase (PHOS)) (Bell et al. 2013). Briefly, 2.75 g of frozen soil was thawed
253 at room temperature and directly homogenized (1 min in a Waring blender) in 200 ml of a
254 sodium acetate buffer solution adjusted to the mean soil pH (5.1 ± 0.7 SD, N= 24) measured
255 at T_0. The soil slurry (800 µL) was then added in technical duplicates to a 96-deep-well
256 microplate with 200 µL of substrates at saturation concentration (V_{max}). For each soil sample,
257 duplicated standard curves (0-100 µM concentration) were prepared by mixing 800 µL of soil
258 slurry with 200 µL of 4-methylumbelliferone (MUB) or 7-amino-4-methylcoumarin (MUC)
259 in 96-deep-well microplates. Plates were incubated at 20°C in the dark (3 h) on a rotary
260 shaker (150 rpm) before centrifugation at 2900 g (3 min). The supernatant (250 µL) was
261 transferred to a black Greiner flat-bottomed plate and fluorescence was measured on a
262 microplate reader (Varioscan Flash, Thermo Scientific) with excitation wavelength set to 365
263 nm and emission set to 450 nm. After correcting for negative controls, potential enzyme
264 activities were expressed as nmol g $soil^{-1}$ h^{-1}. Then, enzymes activities have been summed to
265 represent enzyme activity degrading C-rich (EEC= AG+BG+CB+XYL), N rich (EEN=
266 LAP+NAG), P rich substrates (EEP= PHOS) and total enzymes activity (EEA=
267 EEC+EEN+EEP).

In this study, biomass-specific activity and ecoenzymatic stoichiometry were calculated and used as ecoenzymatic CWM traits proxies (Piton et al. 2019). Biomass-specific activity was obtained by weighting total enzyme activity with microbial biomass-C. This indicator has been considered as a proxy of the average investment in extracellular enzyme activity of one mass-unit of microbe (Allison et al. 2007b, Moorhead et al. 2013, Malik et al. 2019b). Eco-enzymatic stoichiometry was obtained following Sinsabaugh et al. (2009)

Enzymatic C:N ratio (EEC:EEN) = ln(BG):ln(NAG+LAP),

Enzymatic C:P ratio (EEC:EEP) = ln(BG):ln(PHOS)

Enzymatic N:P ratio (EEN:EEP) = ln(NAG+LAP):ln(PHOS).

These ratios were assumed to indicate the direction of the resource acquisition strategy (toward C, N or P).

2.5 Statistical approach

2.5.1 Microbial community composition

A Principal Coordinates Analysis (PCoA) was conducted on the relative abundances of the 27 individual PLFAs at T0, T1 and T2 (full dataset). Then, sample coordinates from the first axis of this PCoA were used as a synthetic variable representing the overall variation in microbial community composition.

2.5.2 Correlations between soil abiotic properties, microbial community composition and ecoenzymatic CWM traits.

Effects of sampling time, microbial community composition (F:B, GP:GN, PCoA-1), soil abiotic properties (Soil-N, pH and moisture) and their interactions on ecoenzymatic stoichiometry (EEC:EEN, EEC:EEP and EEN:EEP) were assessed using mixed effect models with country and plot nested in country as random factors. We also assessed correlations

between microbial composition and soil abiotic properties (Soil-N, pH, moisture) for each sampling time (T0, T1, T2) using mixed effect correlations.

2.5.3 Factor controlling microbial biomass and total enzyme activity

The natural logarithm of the total soil enzyme activity (EEA) can be decomposed in the sum of the natural logarithm of biomass-specific activity ($\ln\left(\frac{\mathrm{EEA}}{\mathrm{Biomass}}\right)$), and natural logarithm of biomass ($\ln(\mathrm{Biomass})$).

$$\ln(\mathrm{EEA}) = \ln(\frac{\mathrm{EEA}}{\mathrm{Biomass}} \times \mathrm{Biomass})$$

$$\ln(\mathrm{EEA}) = \ln\left(\frac{\mathrm{EEA}}{\mathrm{Biomass}}\right) + \ln(\mathrm{Biomass})$$

To assess the relative importance of these 2 components (biomass-specific activity and biomass) in the control of total enzyme activity, regressions of ln(EEA) on ln(Biomass) were fitted for each sampling time. Assuming soil microbial biomass to be the first parameter controlling total enzyme activity, the proportion explained by the model represents the importance of biomass in the control of total enzyme activity in soil while the non-explained variation was attributed to variation in biomass-specific activity.

Piecewise structural equation models (SEM) were used to assess the most important mechanisms driving both parameters (biomass and biomass-specific activity, Figure 2). This method is less sensitive to sample size than standard SEM and enables to implement mixed effect model in the SEM structure (Lefcheck 2016). In such approach, Shipley's test of directed separation (based on a chi-square test (see (Shipley 2000, 2009))) is used to assess model goodness-of-fit, testing if missing paths exist in the model structure. When several models are accepted, information criterion such as Akaike information criterion or Bayesian Information Criterion (BIC) can be used to identify the best model. To obtain the most

parsimonious model we used a three steps selection process, testing a series of potential mechanisms through which soil abiotic environment might influence extracellular enzyme activity, established based on our knowledge of the system (Laughlin et al. 2007, Grace et al. 2015). Firstly, a SEM was fitted with the most parsimonious *a priori* structure, stating that biomass-specific activity was only driven by community composition (Figure 2), and then model fit was assessed. In the case of model rejection (p-val< 0.05), potential missing paths in the SEM structure (such as direct effect of soil abiotic properties on biomass-specific activity, indicating community member's acclimation/adaptation enzyme abiotic stabilization) were evaluated using d-sep test (Shipley 2000, 2003). Secondly, missing paths were added and model fit was newly assessed. Finally, we used a stepwise removal process of non-significant relationships. As De Vries and Bardgett (2016), we tested the effect of each removal using Bayesian Information Criterion (BIC). BIC was used instead of Akaike information criterion because BIC better identified true model in a simulation study with conditions close to our experiment (Hertzog 2018). Each removal was retained if it did not induce a significant increase of BIC criteria (delta BIC< 2) compared to the model with the lower BIC. Global model fit and quality of the final model was verified using Fisher's C test, R^2 of endogenous variables and path significances before starting interpretation, as suggested by Hertzog (2018). Analyses were run under R.3.5.152. (Development Core Team 2013) using packages piecewiseSEM for SEMs (Lefcheck 2016), nlme for mixed effect models (Pinheiro et al. 2017), and ape for PCoA (Paradis and Schliep 2019).

3 Results

3.1 Influence of soil abiotic properties on microbial community composition

A large proportion (43%) of the microbial community composition was explained by the first axis of the PCoA (PCoA-1, Figure 3). PLFAs contributing the most to this axis

337 (coordinates higher than 0.1 or lower than -0.1) were two Gram negative (18:1ω7 and cy17:0)
338 and one Gram positive (a15:0) bacteria which were negatively related to the PCoA-1, whereas
339 two Gram positive (i15:0 and i17:0), one from Actinobacteria (10Me17:0) and one from
340 unclassified bacteria (15:0) were positively associated (Figure 3).

341 The F:B ratio decreased in nutrient rich, alkaline soils under wet condition, as
342 demonstrated by a negative association with soil-N (T0, T2), pH (T0, T2) and moisture (T0,
343 T1, T2) (Table 2). The GP:GN ratio also decreased with nutrient availability (i.e. soil-N at T1
344 and T2), and alkalinity (i.e. pH at T1, and T2). PCoA-1 (Figure 3) showed almost the same
345 behaviour, with negative association with pH (T0, T1, T2), soil-N (T0, T1) and moisture (T0)
346 (Table 2).

347 3.2 Influence of soil abiotic properties and microbial community composition on
348 ecoenzymatic stoichiometry

349 The association between ecoenzymatic EEC:EEN and EEC:EEP and soil abiotic
350 properties highly varied between sampling times (significant interaction between soil
351 properties and time, Figure 4). Negative association between soil-N and EEC:EEN was
352 observed only at T0, whereas EEC:EEN showed a negative association with pH at T0,
353 shifting to positive at T2, and a negative association with moisture at T0 shifting to a positive
354 association at T1 and T2. EEC:EEP ratios showed positive association with soil pH (T1 and
355 T2) and moisture (T2). EEN:EEP showed more constant relationships with soil abiotic
356 properties, increasing with soil-N and pH and decreasing with soil moisture at all sampling
357 times (Figure 4).

358 Conversely, associations between microbial community composition and ecoenzymatic
359 stoichiometry were highly constant between sampling times (Figure 5). More fungal
360 dominated communities (i.e. high F:B) showed an N acquisition strategy as demonstrated by a

positive correlation of F:B with EEN:EEP and a negative one with EEC:EEN (Figure 5). The GP:GN ratio shifted toward a more P-oriented strategy with increasing Gram positive abundance, and toward C and N oriented strategy for Gram negative bacteria as demonstrated by negative associations of GP:GN with EEC:EEP and EEN:EEP (Figure 5). Correlations between GP:GN and EEC:EEN and between F:B and EEC:EEP were not significant. PCoA-1 showed the same association with ecoenzymatic stoichiometry than the GP:GN ratio (data not shown).

3.3 Contributions of microbial biomass and biomass-specific activity to total enzyme activity

The total enzyme activity was significantly correlated with microbial biomass at all sampling times ($p < 0.001$), with R^2 varying from 91% of the EEA variation explained by biomass at T0, to 46% at T1 and 62% at T2, indicating a higher contribution of microbial biomass to potential soil enzyme activity before altered rain regime simulation (T0) and after the recovery period (T2), whereas biomass-specific activity was the most the dominant factor explaining potential soil enzyme activity at the end of the altered rain regime period (T1) (Figure 6).

3.4 Drivers of microbial biomass and biomass-specific activity

Due to their high covariation, PCoA-1 and GP:GN ratio ($R^2 = 0.42$, $p < 0.001$), showed similar responses to soil abiotic factors and had the same effect on biomass-specific activity and microbial biomass. PCoA-1 better explained biomass-specific activity and was therefore conserved in the final structural equation model (Figure 7). At T0 the SEM with full *a priori* structure stating that biomass-specific activity was only driven by microbial community composition, and not by community members' acclimation/adaptation and enzyme stabilization, was accepted ($C6 = 6.05$, $p = 0.42$, $BIC = 88.68$). Then model simplification

based on BIC criterion led to the removal of 6 paths (Figure 7, C18 = 10.31, p = 0.92, BIC = 77.05). At T1 the SEM with full *a priori* structure was rejected (C6 = 18.34, p = 0.005, BIC = 136.47) indicating missing paths in the SEM structure: Community composition was not sufficient to explain biomass-specific activity, suggesting community members' acclimation/adaptation and/or enzyme abiotic stabilization also occurred. D-sep tests showed a missing path between biomass-specific activity and moisture. The addition of this path improved the SEM which was finally accepted (C4 = 2.838, p = 0.59, BIC = 125.51), then model simplification led to the removal of 2 paths (Figure 7, C8 = 11.17, p = 0.19, BIC = 124.75). At T2 the *a priori* model was accepted (C6 = 7.89, p = 0.25, BIC = 126.56), and model simplification based on BIC criterion led to the removal of 4 paths (Figure 7, C14 = 15.75, p = 0.33, BIC = 116.17).

4 Discussion

4.1 Ecoenzymatic CWM traits are tightly linked with microbial community composition along abiotic gradients

Variations of microbial community composition along environmental gradients have been extensively reported (Fierer and Jackson 2006, Allison et al. 2007b, Lauber et al. 2009, De Vries et al. 2012, Fierer et al. 2012a, Ren et al. 2018, Martinez-Almoyna et al. 2019). However, these studies rarely explored how the observed community shifts could explain microbial trait variations (Fierer et al. 2012b, Leff et al. 2015). Community weighted mean (CWM) trait values (the average trait value per unit of biomass within a community) is mostly driven by traits of the dominant species (Lavorel and Garnier 2002, Garnier et al. 2004). Thus, these CWM traits are expected to be associated with the adaptive value of traits along environmental gradients that control community composition changes (Ackerly 2003, Shipley et al. 2006, Laughlin et al. 2018). The first aim of this study was to assess the relationships

409 between microbial community composition and ecoenzymatic CWM traits proxies (Piton et
410 al. 2019) along environmental gradients.

411 Observed associations between soil abiotic properties and community composition were
412 consistent with the literature (De Vries et al. 2006, Ho et al. 2017, Naylor and Coleman-Derr
413 2018), indicating that oligotrophic environments (low resource availability) favour fungi and
414 Gram positive bacteria, while resource-rich conditions were beneficial for Gram negative
415 bacteria (Figure 7 and Table 2). Our trait-based approach showed distinct ecoenzymatic
416 CWM traits associated with these three microbial groups potentially explaining their
417 dominance in oligotrophic and copiotrophic environments respectively. First, fungi were
418 associated with lower biomass-specific activity and their ecoenzymatic stoichiometry
419 suggested their enzyme production to be oriented preferentially toward N acquisition (Figure
420 5). Second, Gram positive bacteria were associated with a higher investment in extracellular
421 enzymes production oriented toward P acquisition (Figure 5). Finally, Gram negative bacteria
422 showed lower investment in enzyme production and a strategy oriented toward C acquisition.
423 The lower biomass-specific potential enzyme activity in more fungal dominated communities
424 (Figure 7) was unexpected since fungi are commonly considered as principal enzyme
425 producers in soils (Sinsabaugh 2005, Romani et al. 2006). However, only hydrolytic enzymes
426 were measured in this study and not oxidative ones, which could have biased this observation.
427 Oxidative enzyme production has being observed in both bacterial and fungal groups (Allison
428 et al. 2007a), but the capacity to produce enzymes degrading lignin is more restricted in
429 microbes than hydrolase production, with important contributions attributed to fungi such as
430 white-rot basidiomycetes (Kirk and Farrell 1987, Boer et al. 2005). Thus, the pattern observed
431 here might also correspond to a shift from a resource acquisition strategy based on hydrolytic
432 enzymes from bacteria, to a strategy more based on oxidative enzymes from fungi. Similar
433 work, crossing hydrolase and oxidase measurements, should shed light on such potential

trade-off. Overall, this result was consistent with the growing idea that bacteria are also important in organic matter degradation (López-Mondéjar et al. 2019). Furthermore, fungal dominance in N-poor soil observed at T0 and T2 (Table 2), associated with higher relative production of N-acquisition enzyme (Figure 5), supports the resource allocation model, which predicts higher investment in N acquisition when N is limiting (Sinsabaugh et al. 1993). Considering also the higher biomass C:N ratio of fungi compared to bacteria (Strickland and Rousk 2010), higher biomass C:N combined with lower EEC:EEN ratio seems to be two response traits associated to low N and high C availability (Mooshammer et al. 2014), likely explaining fungal dominance in such environments. However, direct measurement of microbial biomass stoichiometry would be necessary to fully validate this mechanism.

Decreases of GP:GN and PCoA-1 were associated with variations in ecoenzymatic stoichiometry indicating a shift from P to C acquisition concomitant to a reduction of biomass-specific activity, consistent with our hypothesis 1. The most constant abiotic driver of GP:GN and ecoenzymatic C:P ratio was pH. pH is known to strongly influence P availability, potentially explaining why microbes invest more in P acquisition in acidic soil and shift for C acquisition under neutral conditions where pH constraint on P availability is released (Xu et al. 2017). Our results are also consistent with Gram positive bacteria having a more oligotrophic strategy (Naylor and Coleman-Derr 2018), and depict two traits that might explain their dominance in resource poor and acidic soils: a higher investment in extracellular enzymes to cope with low resource availability (Fontaine et al. 2003, Allison et al. 2007b, Malik et al. 2019b), and a preferential investment in P acquisition to cope with low P availability. Contrastingly, Gram negative bacteria showed a copiotrophic strategy, producing less enzymes (Fontaine et al. 2003), and relying on labile C from plants (Fanin et al. 2018), two traits that might explain their dominance in neutral and resource rich soils. Adding molecular characterization of the microbial communities to our approach would be very

459 valuable to further identify CWM traits associated with community composition at different
460 taxonomic resolution.

461 Ecoenzymatic EEC:EEN ratio was related to soil N at T0, and became more associated to
462 soil moisture at T1 and T2 (Figure 4), while remaining strongly negatively associated with
463 F:B at all sampling times (Figure 5). The relationship between ecoenzymatic EEC:EEP and
464 GP:GN ratio was also more stable through the experiment than the relationship between
465 ecoenzymatic EEC:EEP ratio and soil abiotic factors. This suggests that ecoenzymatic
466 stoichiometry was tightly associated with community composition. Thus, the predicted links
467 between ecoenzymatic stoichiometry with C and nutrient availability (Sinsabaugh et al. 1993,
468 2009) might be limited if other factors such as soil moisture modify community composition.

469 4.2 Total enzyme activity in soils: disentangling mechanisms.

470 The second aim of this study was to assess the relative importance of different
471 mechanisms to control total potential enzyme activity in soils. Our results confirm our
472 hypothesis that biomass primarily controlled total soil enzyme activity under stable
473 conditions, whereas biomass-specific activity (a CWM trait) became the most important
474 factor to predict variations under altered rain regimes (Figure 6). This shows the need for a
475 better understanding of the factors controlling microbial CWM trait variation to model and
476 predict ecosystem level processes, and, especially their transient response to climate changes.
477 Then, we used SEM to disentangle the predominant mechanisms controlling the variation in
478 microbial biomass and biomass-specific activity.

479 4.2.1 Factors controlling microbial biomass

480 Microbial biomass was directly affected by soil-N at T0 (Figure 7). Associated with soil
481 organic matter quantity, higher soil N represents higher amount of resources available for
482 microbes to build up biomass. We also found an important effect through community

composition with F:B ratio having a positive effect on microbial biomass-C (Figure 7), which might be explained by a higher fungal biomass C:N (Strickland and Rousk 2010), a lower nutrient demand or a higher carbon use efficiency (Hodge et al. 2000, Keiblinger et al. 2010, Zechmeister-Boltenstern et al. 2015), indicating a higher capacity to build up microbial biomass-C for a same amount of resources. Conversely, PCoA-1, was negatively related to microbial biomass-C. This link with PCoA-1 was probably not due to a difference in biomass stoichiometry, as PCoA-1 was not associated with F:B ratio. However, oligotrophic communities as indicated by PCoA-1 were likely characterized by a lower investment in biomass production (Figure 1) (Malik et al. 2019a, 2019c). The positive effect of bacterial community composition on biomass-specific activity, translating into a negative effect on biomass suggests that oligotrophic communities invest relatively more C in non-growth products such as enzymes (Malik et al. 2019a). Such trade-off (Figure 1) needs further investigations using molecular and culturing approaches (Malik et al. 2019c, Ramin and Allison 2019). Even if such trade-off across microbial diversity could justify to interpret these SEM paths accordingly (community composition affecting biomass-C), we acknowledge that a feedback might exist between community composition and biomass-C, with high biomass-C potentially influencing community composition by favouring competitive microbes. Thus, our SEM structure should be considered as a potential causal model rather than a proof of a unique causality. To sum up, these results might depict two parallel mechanisms influencing microbial biomass-C through modifications in community composition: 1) a positive effect of fungal abundance through microbial biomass stoichiometry; 2) a negative effect of oligotrophic bacterial community through a higher investment in non-growth products. While the importance of C from microbial origin in soil organic C sequestration is increasingly recognized (Schmidt et al. 2011, Liang et al. 2017), our results provide insights on two potential microbial mechanisms controlling soil C sequestration (Trivedi et al. 2013).

508 4.2.2 Factors controlling biomass-specific activity

509 Studies isolating bacteria and fungi have reported differences in enzymatic traits among
510 microbial taxa (e.g. Lladó et al. 2016, Pierre-Emmanuel et al. 2016), supporting the possibility
511 of a community composition effect on enzyme activity in soils. However, in empirical studies
512 directly measuring potential enzyme activity in soil, the effect of community composition on
513 enzyme activity has rarely been assessed after correction for the microbial biomass effect
514 (Kivlin et al. 2013). Using biomass-specific activity to correct for the biomass effects, our
515 results gave support to this mechanism. Indeed, microbial community composition was the
516 first driver of variation in biomass-specific activity (Figure 7), which showed strong
517 association with F:B, GP:GN and the first PCoA axis used as a proxy of variation in the
518 overall community composition. It is interesting to note that PCoA-1 was a better predictor
519 than a copiotrophic:oligotrophic indicator such as the GP:GN ratio for biomass-specific
520 activity. This invites for further investigations of enzymatic trait variations at a lower
521 taxonomic resolution than broad groups such as fungi, Gram positive and Gram negative
522 bacteria (Ho et al. 2017).

523 Direct positive effects of soil moisture on biomass-specific activity were detected at T1,
524 suggesting other mechanisms than microbial biomass and community composition to control
525 enzyme activity. This direct effect can be attributed to modification of biomass-specific
526 activity without change in community composition induced by physiological acclimation of
527 microbes, and/or evolutionary adaptation of their populations (Schimel et al. 2007, Allison et
528 al. 2014, 2018, Lashermes et al. 2016), and/or enzyme stabilization (Nannipieri et al. 2018).
529 Our statistical approach does not enable us to decouple these mechanisms. However, enzyme
530 turn-over is expected to be down regulated by soil drought, thus increasing the enzyme pool
531 in soil (Steinweg et al. 2012, Kivlin et al. 2013), though we observed a negative effect of dry
532 conditions. Consequently, we attributed the positive effect of soil moisture on biomass-

specific activity to community members' physiological acclimation or evolutionary adaptation of their populations, whereas enzyme stabilization might be only marginal. Although we acknowledge that more studies on enzyme turnover in different environmental conditions (Schimel et al. 2017) are necessary to be fully confident in our interpretation. These community members' acclimation/adaptation suggested by our results indicated a decrease of enzyme production under low soil moisture, and might result from a redirecting of the metabolism from resource acquisition to stress resistance (Schimel et al. 2007, Malik et al. 2019a). This finding stresses the potential importance of physiological acclimation and evolutionary adaptation of microbial traits under climate changes to predict future ecosystem functioning; an open question that urgently needs further experimental and modelling investigation (Romero-Olivares et al. 2015, Allison et al. 2018, Abs et al. 2019).

Overall, our results confirmed that microbial biomass is the first driver of the variation in total enzyme activity in soil under stable conditions, followed by community composition and community members' acclimation/evolution influencing biomass-specific activity, these two last mechanisms becoming especially important under altered climate scenarios. However, it is important to repeat that our study focused on potential and not realized *in situ* enzyme activity, with the latter being the results of both the potential activity and the *in situ* conditions (e.g. temperature, substrate diffusion, pH). Thus, the development of a modelling approaches of realized *in situ* enzyme activity remains essential to fully link with ecosystem functioning (Wallenstein and Weintraub 2008, Steinweg et al. 2012, Allison and Goulden 2017).

5 Conclusion and perspectives

Our study showed that some enzymatic properties (mass-specific activity, enzymatic stoichiometry), considered as proxies of soil microbial CWM traits can be useful to assess the

557 microbial adaptation to environmental variations and the mechanisms controlling ecosystem
558 level total enzyme activity (V_{max}). However, other enzymatic properties should also be
559 considered in the light of the CWM traits concept, such as half saturation constant (K_m),
560 enzyme efficiency (K_{cat}), enzyme temperature sensitivity (Q_{10}) and pH optimum. Studies
561 assessing the response to environmental variations of these ecoenzymatic CWM traits (e.g.
562 Bárta et al. 2014 and German et al. 2012) and their relationships with microbial community
563 composition (e.g. Tischer et al. 2015, Puissant et al. 2019), are highly valuable to move
564 forward our understanding of microbial adaptation, community assembly and their links to
565 ecosystem functioning.

566 Using a functional trait framework to design our study and interpret our results, we bring
567 new insights on the mechanisms controlling total enzyme activity in soils. Our results indicate
568 a tight association between microbial community composition and ecoenzymatic traits with
569 important consequences for total enzyme activity at the ecosystem level. Our results also
570 stress the relevance of approaches disentangling the effect of biomass and biomass-specific
571 activity on microbially-mediated ecosystem processes (Billings and Ballantyne 2013, Kivlin
572 et al. 2013). We argue that empirical studies could develop a more mechanistic understanding
573 by implementing this framework. Advancing our understanding of the roles of microbial traits
574 in physiological acclimation, evolutionary adaptation, community composition changes, and
575 ecosystem functioning should bring relevant insights to improve emerging microbial trait
576 based models (Allison 2012).

577 ## 6 Authors' contributions

578 AF, KH, PS, GBDD and JCC designed the experiment together with other partners of the
579 ECO-SERVE project. PMS, EN, FR and PS carried out the experiment in the Laboratory of
580 Soil Ecology and Ecotoxicology of the University of Coimbra. All authors participated in
581 sampling. GP, NL, JCC, AF conducted the enzymatic assays. KH measured the PLFA. LMG

conducted the soil chemistry analyses. GP run all the statistical analyses, wrote the first draft of this paper and edited it based on significant comments from AF, LMG, NL, KH, GBDD and JCC. All authors gave final approval for publication.

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904 Figures

905

906 Figure 1 Response-effect trait model (Lavorel and Garnier 2002) presenting linkages between
907 response traits (organisms response to environmental changes) and effect traits (organisms
908 effect on ecosystem functioning), adapted for soil microbial community, demonstrating the
909 central position of ecoenzymatic traits (enzyme production for C, N and P acquisition) and
910 biomass stoichiometry (C/N/P) in evolutionary and/or physiological trade-off (dotted arrows)
911 affecting fitness and community composition, and microbial community effect on ecosystem
912 functioning.

913

914 Figure 2 *A priori* model tested using piecewise-SEM stating: soil abiotic factors influence on
915 microbial community composition (arrow 1) and community composition effect on biomass-
916 specific activity (arrow 2) explained by the difference in enzyme production between taxa ;
917 community composition effect on biomass-C (arrow 3) explained by stoichiometry, growth or
918 carbon use efficiency difference between taxa; direct abiotic effect of soil abiotic properties
919 which influence amount and availability of resources (arrow 4); potential cost of enzyme
920 production for biomass-C build up (arrow 5; direct effect of soil abiotic properties on
921 biomass-specific activity representing either a community members' acclimation/evolution
922 (change in enzyme production without modification of community composition) or enzyme
923 abiotic stabilization (reduction of enzyme turnover in soil induced by change in abiotic
924 environment, arrow 6). Arrow 6 (grey) was initially not included in the model and only added
925 according to d-sep test (Shipley 2000, 2003, Lefcheck 2016). ε represent error terms. One-
926 headed arrows represent causal relationships; double-headed arrows represent free
927 correlations.

928

929 Figure 3 PCoA plot of the 27 identified PLFAs from the 3 sampling times (T0, T1 and T2)
930 representing the overall variation of the microbial community composition. Colours represent
931 microbial groups.

932

933 Figure 4 Correlations between ecoenzymatic stoichiometry and soil abiotic properties (Soil-N,
934 moisture and pH) at different sampling times (T0: green, T1: red, T2: blue) and significance
935 tested with mixed effect model using country as random factor. NS: $p > 0.05$, *: $p < 0.05$, **:
936 $p < 0.01$, ***: $p < 0.001$. Point shapes represent the different rain regimes (square= dry,
937 diamond= normal, triangle= intermittent, wet= circle).

938

939 Figure 5 Correlation between ecoenzymatic stoichiometry and community composition (Gram
940 positive: Gram negative (GP:GN) and Fungal : Bacterial (F:B) ratios) at different sampling
941 time (T0: green, T1: red, T2: blue) and significance tested using mixed effect model using
942 country as random factor. Correlations between GP:GN and EEC:EEN and between F:B and
943 EEC:EEP are not presented because they were not significant. NS: $p > 0.05$, *: $p < 0.05$, **: $p <$
944 0.01, ***: $p < 0.001$. Point shapes represent the different rain regimes (square= dry, diamond=
945 normal, triangle= intermittent, wet= circle).

946

947 Figure 6 Correlation between the natural logarithm of the total enzyme activity and the natural
948 logarithm of microbial biomass-C for the three sampling times (T0: green points solid line,
949 T1: red points, dotted line T2: blue points dashed line), left panel. Proportion of total enzyme
950 activity explained by biomass-C (R^2 of the correlation between natural logarithm of the

951 enzyme activity and the natural logarithm of microbial biomass) and biomass-specific activity
952 (variation not explained by biomass-C), right panel.

953

954 Figure 7 Final structural equation models at T0, T1 and T2. Hypothetical causal relationships
955 are represented by one-headed arrows and free correlations with double-headed arrows.
956 Arrow width represents standardized effect size. Solid line represents positive effect and
957 dashed line negative effect. Black arrows represent significant effect and grey arrow non-
958 significant effect conserved during selection process.

959

960 Tables

961 Table 1. Characterization of the field sites from which intact soil cores were collected and their contrasting management. MAT= Mean annual

962 temperature, MAP= Mean annual precipitation, N= Nitrogen. See Lori et al. (2020) for more details.

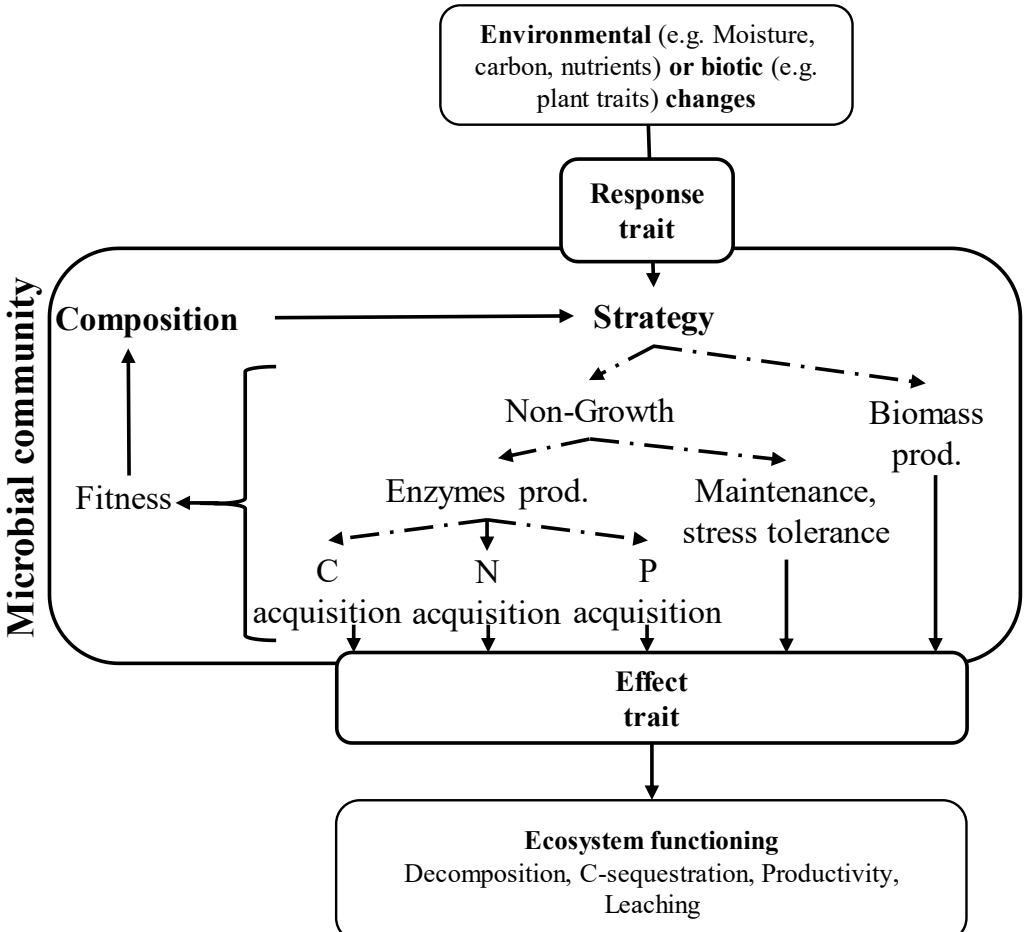
Country (coordinates)	Land use	N Fertilizer (average N kg ha^{-1} $year^{-1}$)	MAT, MAP	Texture	pH	SOM	WHCmax
Switzerland 47°30′N 7°33′E	Grassland in rotation	Ecological-intensive: Slurry (120) Conventional-intensive: Synthetic (140)	9.7 °C, 791 mm	Silt / Silt Loam	5.01 (±0.12)	4.15 % (±0.67)	58.91 % (1.89)
France 45°07′N 5°31′E	Mountain grassland	Ecological-intensive: Cow manure (30) Conventional-intensive: Cow manure (70)	7.2 °C, 1483 mm	Sandy Loam / Loam	5.71 (±0.86)	9.34 % (±2.46)	90.92 % (±8.93)
Portugal 38°42′N 8°19′W	Grassland in agroforest	Ecological-intensive: None (0) Conventional-intensive: Synthetic (56)	16.5 °C, 1093 mm	Sandy Loam	4.62 (±0.35)	3.55 % (±0.64)	39.18 % (±4.45)

963

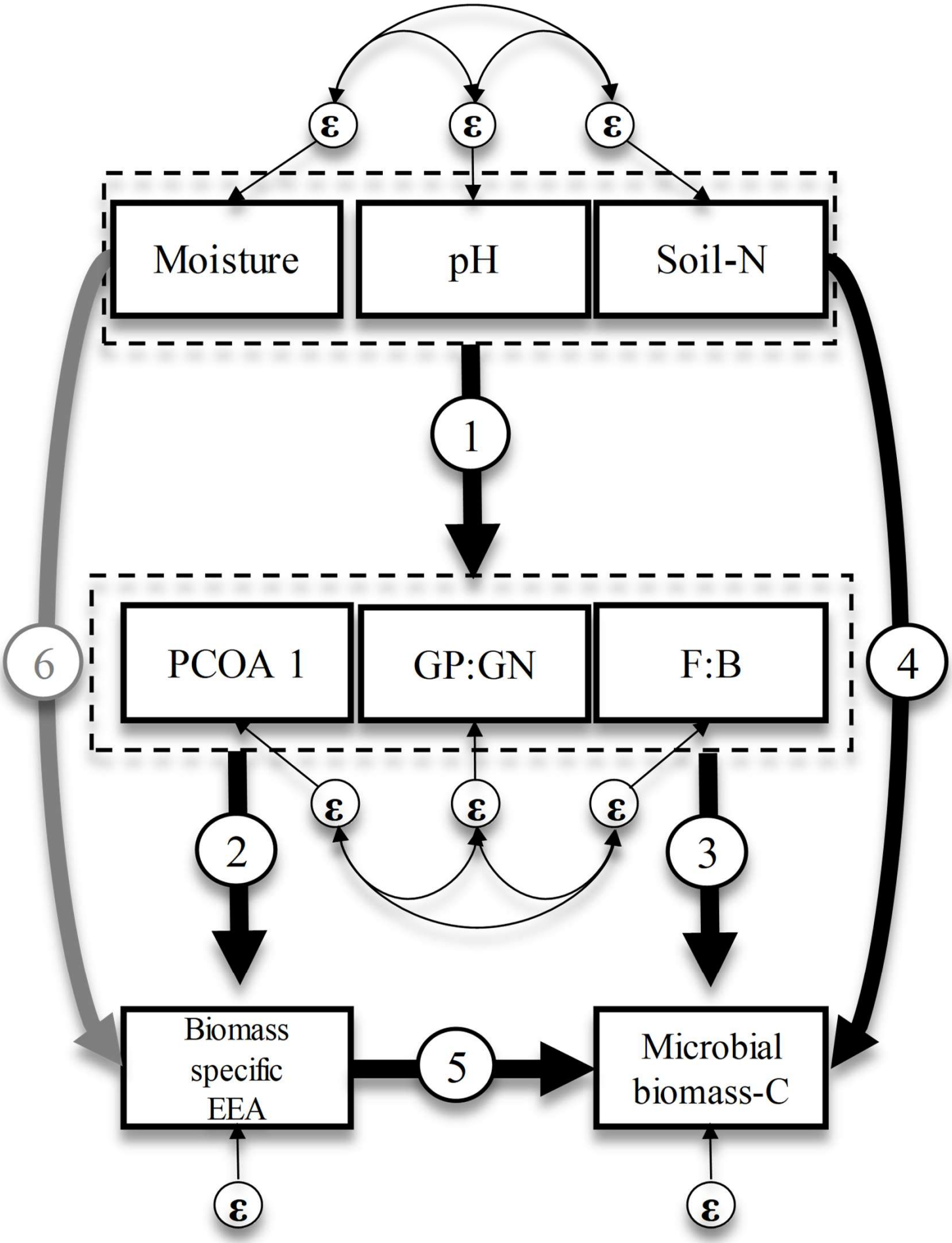
964

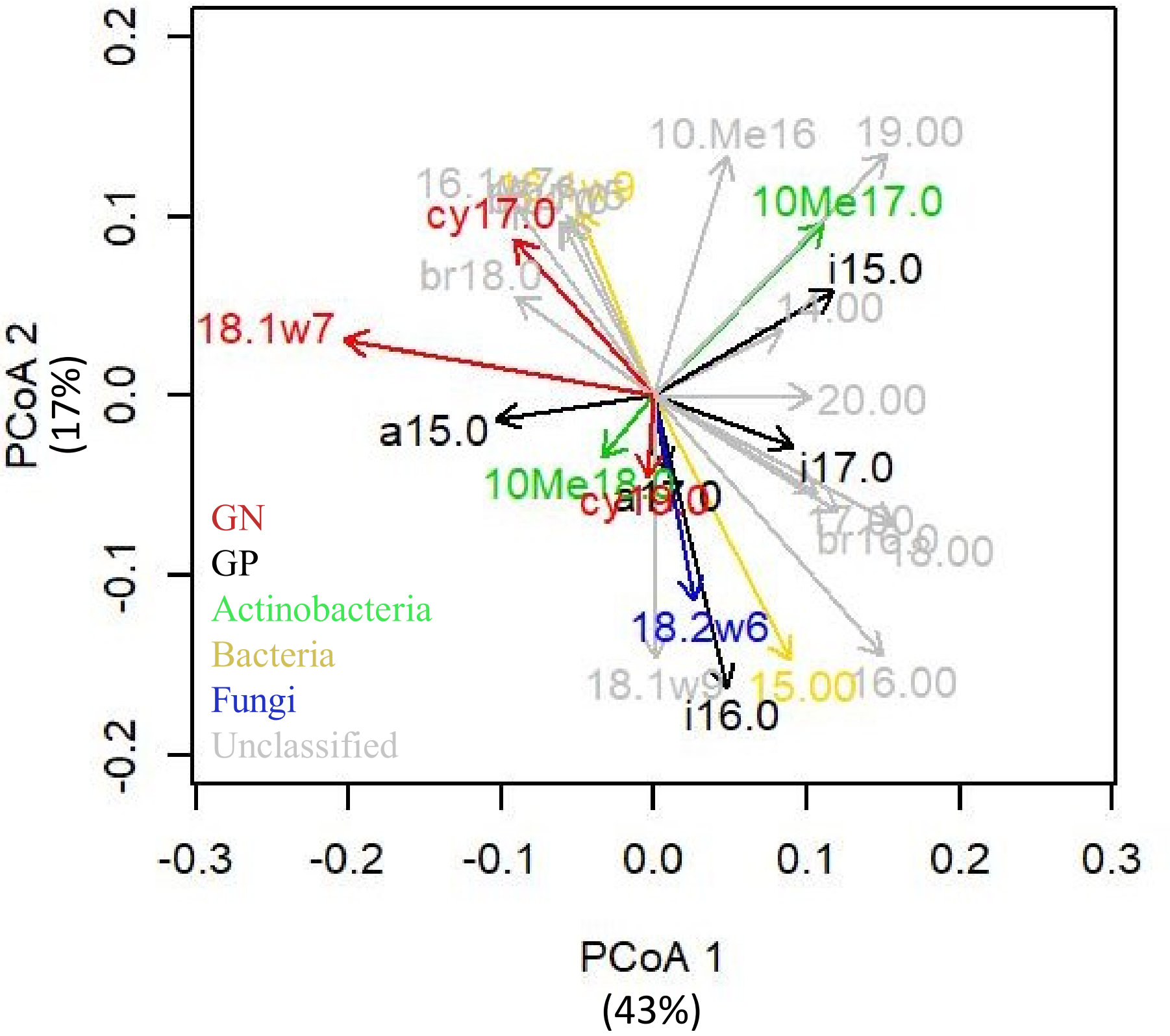
Microbial community composition	Time	Soil abiotic properties					
		Soil-N		pH		Moisture	
		Coef	p	Coef	p	Coef	p
F:B	T0	**-0.62**	**<0.01**	**-0.40**	**0.05**	**-0.68**	**<0.01**
	T1	-0.21	0.08	-0.17	0.13	**-0.25**	**0.01**
	T2	**-0.28**	**0.02**	**-0.33**	**<0.01**	**-0.36**	**<0.001**
GP:GN	T0	-0.18	0.17	-0.14	0.22	**-0.54**	**0.01**
	T1	**-0.41**	**<0.001**	**-0.26**	**<0.01**	**-0.20**	**0.03**
	T2	**-0.22**	**0.03**	**-0.24**	**<0.001**	**-0.21**	**0.05**
PCoA-1	T0	**-0.26**	**<0.01**	**-0.18**	**0.03**	**-0.57**	**<0.001**
	T1	**-0.40**	**<0.001**	**-0.43**	**<0.001**	0.09	0.30
	T2	-0.12	0.14	**-0.39**	**<0.001**	-0.02	0.83

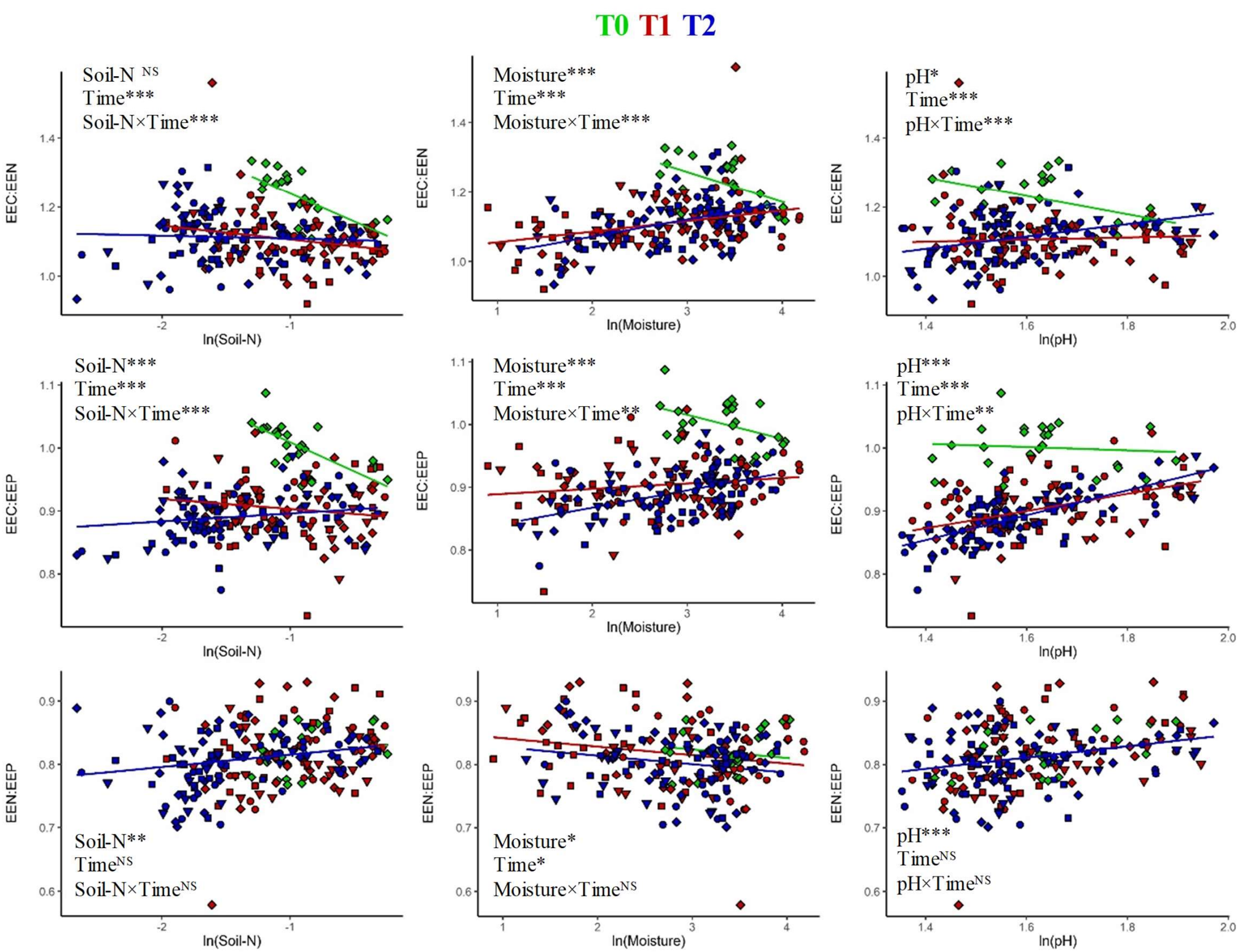
965 Table 2 Bivariate correlations between microbial community composition and soil abiotic properties
966 at T0, T1 and T2. Coef= standardized coefficient, p=correlation p-value. Values in bold indicate a
967 significant p-value.

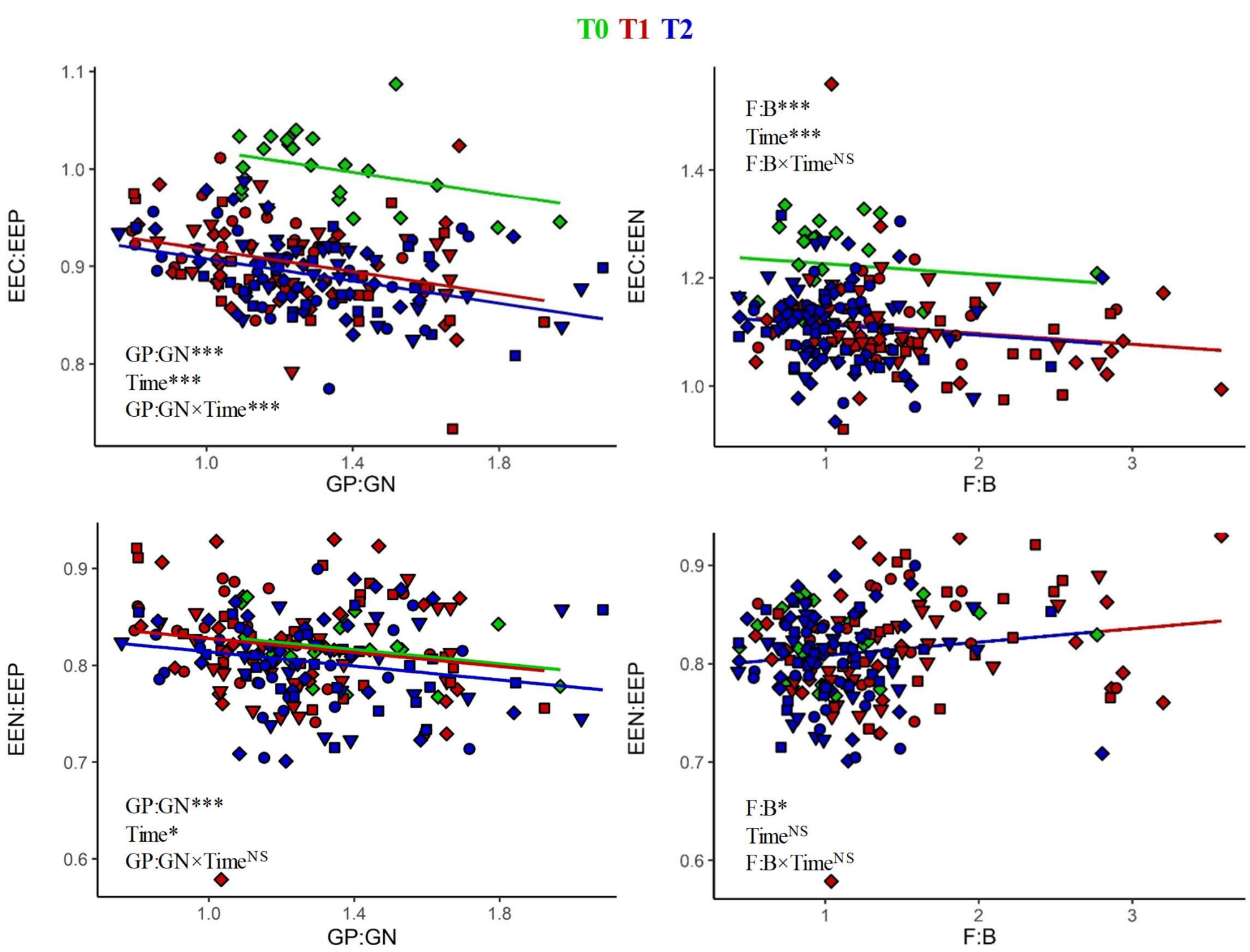

Environmental (e.g. Moisture, carbon, nutrients) **or biotic** (e.g. plant traits) **changes**
Response trait
Microbial community
Composition
Strategy
Non-Growth
Biomass prod.
Fitness
Enzymes prod.
Maintenance, stress tolerance
C acquisition
N acquisition
P acquisition
Effect trait
Ecosystem functioning
Decomposition, C-sequestration, Productivity, Leaching

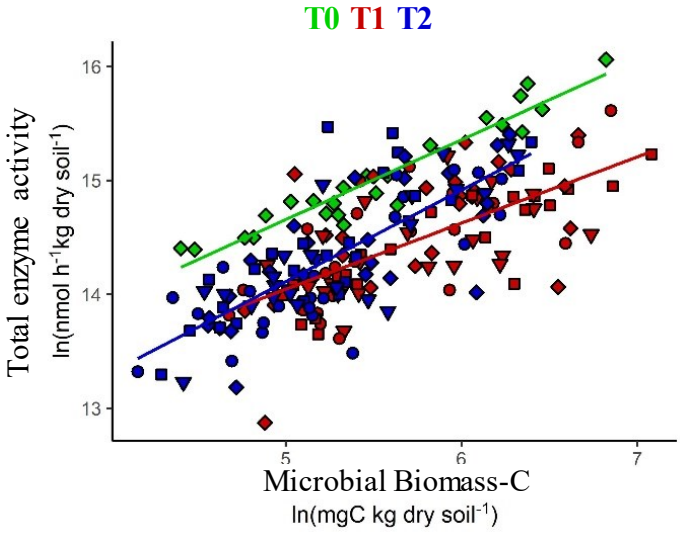
a priori model tested with SEM

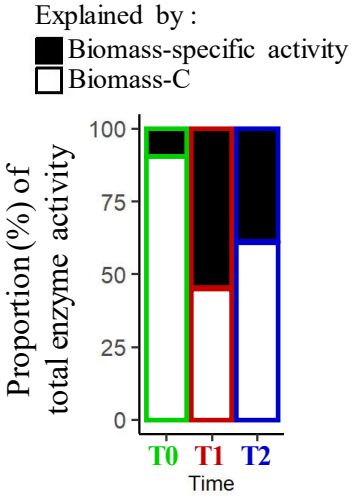


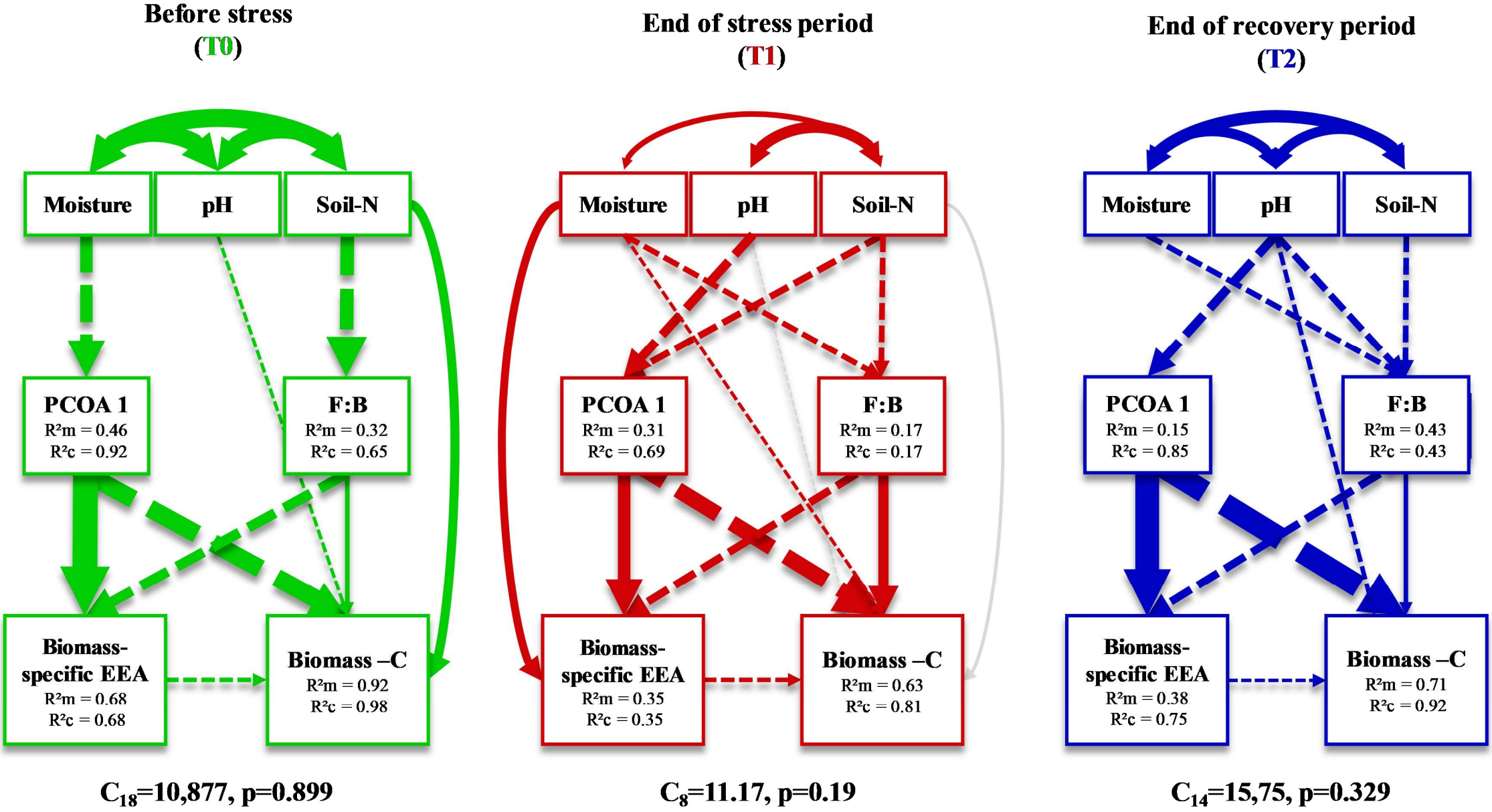

PCoA 2
(17%)
PCoA 1
(43%)
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3
10.Me16
19.00
cy17.0
10Me17.0
br18.0
i15.0
18.1w7
20.00
a15.0
i17.0
10Me18.0
cy18.0
a18.0
br18.00
18.2w6
18.1w9
15.00
16.00
i16.0
GN
GP
Actinobacteria
Bacteria
Fungi
Unclassified


T0 T1 T2
Soil-N NS
Time***
Soil-N×Time***
Moisture***
Time***
Moisture×Time***
pH*
Time***
pH×Time***
Soil-N***
Time***
Soil-N×Time***
Moisture***
Time***
Moisture×Time**
pH***
Time***
pH×Time**
Soil-N**
TimeNS
Soil-N×TimeNS
Moisture*
Time*
Moisture×TimeNS
pH***
TimeNS
pH×TimeNS
EEC:EEN
EEC:EEP
EEN:EEP
ln(Soil-N)
ln(Moisture)
ln(pH)


T0 T1 T2
EEC:EEP
GP:GN
GP:GN***
Time***
GP:GN×Time***
EEC:EEN
F:B
F:B***
Time***
F:B×Time^NS
EEN:EEP
GP:GN***
Time*
GP:GN×Time^NS
EEN:EEP
F:B
F:B*
Time^NS
F:B×Time^NS


T0 T1 T2
Total enzyme activity
ln(nmol h-1kg dry soil-1)
16
15
14
13
5
6
7
Microbial Biomass-C
ln(mgC kg dry soil-1)


Explained by :
Biomass-specific activity
Biomass-C
Proportion (%) of total enzyme activity
100
75
50
25
0
T0 T1 T2
Time


Before stress
(T0)
Moisture
pH
Soil-N
PCOA 1
R²m = 0.46
R²c = 0.92
F:B
R²m = 0.32
R²c = 0.65
Biomass-specific EEA
R²m = 0.68
R²c = 0.68
Biomass –C
R²m = 0.92
R²c = 0.98
C18=10,877, p=0.899
End of stress period
(T1)
Moisture
pH
Soil-N
PCOA 1
R²m = 0.31
R²c = 0.69
F:B
R²m = 0.17
R²c = 0.17
Biomass-specific EEA
R²m = 0.35
R²c = 0.35
Biomass –C
R²m = 0.63
R²c = 0.81
C8=11.17, p=0.19
End of recovery period
(T2)
Moisture
pH
Soil-N
PCOA 1
R²m = 0.15
R²c = 0.85
F:B
R²m = 0.43
R²c = 0.43
Biomass-specific EEA
R²m = 0.38
R²c = 0.75
Biomass –C
R²m = 0.71
R²c = 0.92
C14=15,75, p=0.329