Does plant community plasticity mediate microbial homeostasis?

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- Species cover: Arctic Data Center Entry
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- Soil microbial biomass C, N and P; extracellular enzyme activity, soil extractable C, N and P; soil total C, N and P, in g m^{-2} (Appendix Table 1) and µg g^{-1}: Arctic Data Center Entry
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Abstract

Microbial homeostasis - constant microbial element ratios along resource gradients – is a core ecological tenet, yet not all systems display homeostasis. We suggest investigations of homeostasis mechanisms must also consider plant-microbial interactions. Specifically, we hypothesized that ecosystems with strong plant community plasticity to changing resources will have homeostatic microbial communities, with less microbial resource cost, because plants reduce variance in resource stoichiometry. Using long-term nutrient additions in two ecosystems with differing plant response we fail to support our hypothesis because although homeostasis appears stronger in the system with stronger plant response, microbial mechanisms were also stronger. However, our conclusions were undermined by high heterogeneity in resources, which may be common in ecosystem-level studies, and methodological assumptions may be exacerbated by shifting plant communities. We propose our study as a starting point for further ecosystem-scale investigations, with higher replication to address microbial and soil variability, and improved insight into microbial assimilable resources.

Introduction

Organismal homeostasis is defined as consistency in element composition despite fluctuations in environmental resource availability (Sterner & Elser, 2002). This is a core tenet of ecological stoichiometry, a conceptual framework that explains how element proportions drive processes in organisms and ecosystems. In plant-soil-microbial systems, microbial homeostasis determines rates of decomposition, nutrient retention and biomass production (Zechmeister-Boltenstern *et al.*, 2015), processes that predict food security, fertilizer pollution, and carbon storage (Paustian *et al.*, 2016). Uncertainty in these predictions arise because microbial homeostasis can be both strong or weak, in other words, microbial C:N:P is sometimes invariant, and sometimes varies with resource C:N:P. Despite increasing attention to the mechanisms that support microbial homeostasis, it remains unclear what causes this variation.

The current paradigm for exploring microbial homeostasis was nicely outlined by Mooshammer et al. (2014), with a four-option mechanistic framework describing how microbial communities can respond to the stoichiometric imbalance between their biomass and their resources at the individual and or community level. Microbes may: (1) store C, N or P or shift community structure to match their biomass composition towards their resource; (2) mobilize needed resources by enhancing extracellular enzyme activity (EEA); (3) alter element use efficiencies (the ratio of the investment of an element in growth vs. the total uptake of this element) by excreting nutrients in excess; or (4) alter resource pools via inputs of nutrients external to the measurement system, such as by N-fixing prokaryotes or fungal hyphae. There was an earlier focus in modelling and experimental research on EEA as the dominant mechanism (Sinsabaugh *et al.*, 2009), while more recent studies suggest that changing nutrient use efficiency is the primary mechanism (Mooshammer *et al.*, 2014; Fanin *et al.*, 2016; Manzoni *et*

69 *al.*, 2017). Here, we propose that mechanistic studies of microbial homeostasis at the ecosystem
70 scale – with associated higher complexity of interactions - should also consider plant community
71 mechanisms that alter the soil resource pool.
72 Changing plant community structure may influence nutrient outputs, inputs and the
73 associated microbial community, three mechanism that could change how the microbial
74 community maintains, or fails to maintain homeostasis. *Nutrient outputs:* plant functional types
75 take up nutrients in different ratios (McLaren & Turkington, 2010). *Nutrient inputs*: changing
76 soil resources changes the stoichiometric ratio of individual plant tissue (Shaver & Chapin 1980)
77 and the community averaged plant tissue stoichiometry, through shifts in plant functional group
78 composition (Guiz *et al.*, 2016). Individual and community tissue stoichiometry alters litter and
79 root exudate stoichiometry, and the litter:exudate ratio of these inputs, the latter which may
80 further change microbial mechanisms of C and nutrient acquisition (Sokol *et al.*, 2018). *Plant-*
81 *microbe associations*: not all microbial populations maintain homeostasis (Danger *et al.*, 2016)
82 so when plant community shifts are associated with microbial community shifts, this may lead to
83 changes in the preferred mechanisms or ability to maintain homeostasis.
84 Soil type is the dominant control on microbial community structure and activity, with
85 both plant community and resource supply secondary controls (Fierer, 2017). Studies that
86 examine microbial efforts to maintain homeostasis under various resource regimes have been
87 most commonly conducted with both soil type and plant communities varying (Cleveland &
88 Liptzin, 2007; Sinsabaugh *et al.*, 2009; Nottingham *et al.*, 2015; Fanin *et al.*, 2016; Tipping *et*
89 *al.*, 2016), or in mesocosms on the same soil type with the same (or no) plant community
90 (Joergensen & Scheu, 1999; Heuck *et al.*, 2015; Pinsonneault *et al.*, 2016; Zhou *et al.*, 2017). In
91 ecosystem-scale investigations of homeostasis, both the microbial community and the plant

92 community respond to the changes in resource availability, although not always in tandem, while
93 soil type usually remains constant. We are not aware of examinations of microbial homeostasis
94 using experimental nutrient additions resulting in changing plant functional groups on the same
95 soil type, despite that this is a likely outcome of enhanced nutrient pollution in natural
96 ecosystems (Dormann & Woodin, 2002; Suding *et al.*, 2005; Xia & Wan, 2008).
97 We addressed this research gap by investigating microbial homeostasis and potential
98 mechanisms – including the role of plants - for ecosystem response to stoichiometric imbalance
99 in long-term fertilization experiments in two dominant, close-proximity (< 2 km separation),
100 upland Arctic tundra ecosystems: Moist Acidic Tundra (MAT) and Moist Non-acidic Tundra
101 (MNT). Despite similar vegetation functional groups, vegetation community response to
102 fertilization differs between the sites. The MAT has responded to fertilization with large
103 increases in *Betula nana*, a deciduous shrub known for its plastic response to nitrogen and
104 phosphorus additions (Bret-Harte *et al.*, 2002). In contrast, the MNT initially responded to
105 fertilization with increases in a variety of functional groups, particularly forbs and grasses
106 (Hobbie *et al.*, 2005), and in the longer term responded primarily with reductions in moss and
107 few changes in vascular plants (McLaren & Buckeridge, 2019). We use these two sites to test the
108 hypothesis that a large plant community response to fertilization can minimize microbial effort
109 while still maintaining microbial homeostasis across a steep gradient of resource supply.
110
111 **Site Description and Methods**
112 **Study Site.** The study was conducted at the Arctic LTER site at Toolik Lake in northern Alaska,
113 USA (68°38'N and 149°43'W, elevation 760 m) in the MAT and MNT (described previously in
114 Buckeridge and McLaren 2019) which are dominant ecosystems of the Alaskan tundra. The two

ecosystems differ based on age and acidity: the MAT site is on older substrate (50k – 120k y) with a pH = 3-4, and the MNT is on younger substrate (11.5-25k y), with a neutral pH.

Experimental Design. We sampled existing long-term fertilization experiments established and maintained by the Arctic LTER in both vegetation types. Fertilization treatments in both experiments represent a full-factorial addition of N (10 $g/m^2/yr$ as NH_4NO_3) and P (5 $g/m^2/yr$ as P_2O_5), with fertilizer applied annually in pellet form following snowmelt (early June) for 26 years (MAT, established in 1988) and 16 years (MNT, established in 1997). From each experiment we sampled a single 5 x 20 m plot from each of the three treatments (N, P, N+P) and the control, from each of four (MAT) or three (MNT) replicate blocks.

Vegetation and soil sampling. Aerial percent cover of mosses, lichens, litter and all vascular plant species was visually estimated in each treatment plot in mid-July 2013, within eight -$1m^2$ adjacent quadrats in each plot. Vole litter was assigned visually as the haying/nesting activities of small mammalian herbivores, primarily *Microtus oeconomus* and *M. miurus.* A ca. 10cm x 10cm column of soil was collected from each MAT and MNT plot to the depth of the permafrost in early July 2013. All organic horizons were <20cm deep and were separated into the upper organic (0 – 5cm depth) and lower organic (>5cm depth) layers. Soils were homogenized and all large roots (>1mm diameter) removed in the field lab. Soil was then partitioned for the analyses below, frozen at -20^oC and shipped for analyses.

Soil and microbial biomass extraction and analysis. Field-moist and thawed soil samples (10 g) were shaken with 40 ml of ultra-pure water, or with water plus $CHCl_3$ (Fierer *et al.*, 2003). Extractable organic C (EOC) and total N (ETN) and PO_4-P contents in the $CHCl_3$ and non-$CHCl_3$ extracts were determined as described previously (McLaren & Buckeridge 2019).

Soil microbial extracellular enzyme analysis. We assayed for the activity of three hydrolytic enzymes that release C, N and P at the terminal stages of organic matter decomposition: cellulose-degrading beta-glucosidase (BG), chitin-degrading N-acetyl-glucosaminidase (NAG) and phosphatase (AP), using standard methods, as described previously (McLaren & Buckeridge 2019).

Data analysis and statistical models. The two ecosystems have different lengths of time under experiment and were thus evaluated separately and the results qualitatively compared. For all soil analyses, data from both organic horizons (upper and lower) were pooled, with the values weighted by the depth of each horizon. Microbial biomass C, N and P flushes (hereafter, MBC, MBN and MBP) were calculated as the difference between EOC, ETN or PO_4-P in $CHCl_3$ and non-$CHCl_3$ extracts, with no correction factor for incomplete $CHCl_3$-release applied. For potential enzyme activity (EEA), for each substrate, we measured the background fluorescence of soils and substrate and the quenching of MUB by soils, and used standard curves of MUB to calculate nmol of substrate hydrolysed per hour per g of soil. Vegetation data were analysed as relative percent cover for each species and also for functional groups (calculated as the sum of all component species).

For each ecosystem, we used variations on the metric H for determining homeostasis:

$$H = \frac{1}{m},$$

where m is the slope of $\log_e C{:}N_R$ or $\log_e C{:}P_R$ (resources) versus $\log_e C{:}N_B$ or $\log_e C{:}P_B$ (microbial biomass) (Cui *et al.*, 2018). Strictly homeostatic organisms have an H of infinity, which presents analytical problems, and so the regression slope 1/H was used in its place (as in Persson et al. 2010). If the regression slope is not significant, the organisms are considered homeostatic (Persson *et al.*, 2010).

CUE was estimated from the stoichiometry of the organic matter, microbial biomass and extracellular enzyme activity (Sinsabaugh *et al.*, 2016) for both C:N and C:P:

$$CUE_{c:x} = CUE_{max}[\frac{S_{c:x}}{(S_{c:x} + K_x)}],$$

where the half-saturation constant K_X was 0.5, and $CUE_{max,}$ the upper limit for microbial growth efficiency based on thermodynamic constraints, was 0.6 (Sinsabaugh *et al.*, 2016). $S_{C:X}$ represents the offset by extracellular enzyme activity of the imbalance between the elemental composition of available resources and the composition of microbial biomass:

$$S_{c:x} = \left(\frac{1}{EEA_{c:x}}\right)\left(\frac{MB_{c:x}}{L_{c:x}}\right),$$

where L is the elemental composition of the substrate consumed (TOC, TN or PO_4). We used TOC, TN and PO4 to represent substrates rather than total soil C, N or P as water-soluble nutrients likely represent a more sensitive measure of the soil substrate driving microbial activity (Mooshammer et al. 2014).

Our assays included potentials (EEA) or calculations based on potentials (CUE), in addition to comparison between different ecosystem processes. Therefore, we calculated the effect of long-term nutrient addition on extracellular enzyme activity, CUE and vegetation composition as the natural log of the response ratio (fertilized/control) for each block (Cusack *et al.*, 2010). Significant effect sizes were determined based on their difference from zero (α = 0.05).

Results

Microbial biomass C:N:P homeostasis was evident in the MAT ecosystem, for both C:N and C:P (Fig. 1 a & b) and in the MNT system, for C:P (Fig. 1 d). In all three of these results, homeostasis is assumed because the slopes of the organism: resource stoichiometries are not

different from zero. In contrast, in the MNT, microbial biomass C:N declined with resource C:N, indicating no microbial homeostasis in this system: microbes reduced their biomass N concentration in N-rich soils (Fig 1c).

Response ratios assess resource treatment relative to control, but also allow comparison between ecosystem-level effects. Our combined plant and microbial analyses indicate that, in both ecosystems, plant community changes generated since the inception of the nutrient addition have a higher response ratio than microbial EEA or CUE (Fig 2a-f).

In the MAT with the addition of N alone, microbial CUE-N increased (Fig 2b), deciduous shrubs increased in relative abundance, and evergreens decreased (Fig. 2c). With the addition of P alone, microbial NAG activity declined (Fig 2a), microbial CUE-P increased (Fig 2b), there was a trend for deciduous shrubs to decline and vole litter increased (Fig. 2c). With the addition of both N and P, all responses were in the plant community: deciduous shrubs and forbs increased, and mosses, graminoids and evergreens declined in relative abundance (Fig. 2c).

In the MNT with the addition of N alone, there was a trend for NAG to increase (Fig. 2d) and graminoid relative abundance to increase (Fig. 2f). With P addition alone, there was no detected microbial homeostatic response and only a trend for moss abundance to decline (Fig. 2f). With the addition of both N and P, all responses were again in the plant community: mosses declined and vole litter increased (Fig. 2f).

Discussion

The mechanistic portion of our hypothesis, that a strong plant response would reduce the variation in the stoichiometry of resources also reducing the microbial efforts to maintain homeostasis, was not supported. The ecosystem with the stronger plant community response

204 (MAT) also had a wider range of soil resource stoichiometry (Fig. 1) and there was no indication
205 that microbial effort towards stoichiometry was lower in this community (Fig 2). Instead, the
206 microbial efforts to maintain homeostasis, as measured by changes in CUE and potential enzyme
207 activity, were generally low in both ecosystems (compared to the plant response) but significant
208 in the ecosystem with the stronger plant response (MAT). In the MAT there was an increase in
209 CUE (C:N) with N additions and CUE (C:P) with P additions, supporting microbial shifts in use
210 efficiencies with resource shifts (Mooshammer *et al*. 2014). There was also a decrease in NAG
211 production (extracellular enzyme which supports microbial N acquisition) with P additions in the
212 MAT, possibly in response to decreased N requirements for P uptake or enzyme synthesis.
213 We present our lack of support for the mechanistic hypothesis not as confirmation that
214 these mechanisms are not important, but as a call to further research, in plant-soil systems with
215 either less variance or larger replication. Inherent variability in the microbial C:N, or especially
216 C:P ratios, temper our conclusions of homeostasis in both ecosystems, but a lack of ecosystem-
217 level research leaves us unable to conclude whether this variability is unusual. We are not aware
218 of other studies examining mechanisms for microbial homeostasis that occur in environments
219 where the both soil resource variability and plant composition vary strongly between areas on the
220 same soil type. Studies in which both plant communities and soil resources vary in concert
221 include either shifts in vegetation composition across strong environmental gradients (e.g.
222 elevational gradients – Nottingham et al. 2015) or only subtle changes in vegetation communities
223 with changing soil resources (e.g. shifts within a plant functional group – Griffiths et al. 2012).
224 Our two long-term study systems have a replication level (n=3 or 4) that is not unusually low
225 compared with other similar long-term studies, and this level of replication has been sufficient
226 for numerous investigations with significant results over the past three decades (Chapin *et al.*,

227 1995; Mack *et al.*, 2004; Hobbie *et al.*, 2005; Koyama *et al.*, 2013; McLaren & Buckeridge,
228 2019). Nonetheless, higher levels of replication may be necessary for studies that examine
229 coupled responses of vegetation and soil communities. Where possible, higher levels of
230 replication would also improve mechanistic insight into homeostasis, for instance to move
231 beyond binary (yes/no) responses and instead allow researchers to assess the degree of variation
232 in biomass stoichiometry, in different systems.

233 We found that the MAT ecosystem with a stronger plant biomass and community
234 response (Fig. 2, McLaren and Buckeridge 2019), showed microbial homeostasis for both N and
235 P whereas microbes in the MNT, with a weaker plant response, showed non-homeostatic
236 behaviour for N and homeostasis only for P. However, high heterogeneity in fertilized natural
237 systems may also make currently used metrics of homeostasis inappropriate. In the MAT, the
238 homeostatic relationship for P was much weaker than that for N due to the very high variability
239 in microbial C:P irrespective of resource C:P. In the MNT, we also saw very high variability in
240 microbial C:P. According to frequently used metrics for homeostasis, these three relationships
241 (MAT C:N and C:P and MNT C:P) are defined as homeostatic – variations in soil element ratios
242 do not significantly affect microbial biomass element ratios because the slopes of the regressions
243 (Fig. 2) do not significantly differ from zero (Persson et al. 2010). However, this metric of
244 homeostasis does not distinguish between strict homeostasis (changes in resource stoichiometry
245 has no influence on organism stoichiometry (Sterner and Elser 2002)) and those where the
246 microbial stoichiometry is highly variable but also not dependent on resource stoichiometry.
247 Persson et al. (2010) used a meta-analysis approach to examine whether studies with non-
248 significant slopes may have been misclassified as homeostatic, by using the residual variation in
249 the datasets that had a significant regression fit (i.e., classified as non-homeostatic, as with Fig

250 2b-d) as an estimate of background variation, and then comparing this with the variation in the
251 homeostatic datasets. With this approach, Persson et al. (2010) determined that for most of the
252 species they examined, the homeostatic relationships were correctly classified. However, in
253 studies with a more limited data set such as ours, for which estimating background variation in
254 this way is difficult, we propose an alternate index of variation (i.e. including a minimum R^2)
255 should be used to define homeostasis. Although the large spread in the resource C:P in our study
256 should be ideal for such a determination, many more data points are needed and are unavailable
257 in this or most long-term experimental manipulations. Therefore, we do not believe that our data
258 provide sufficient evidence of homeostasis.

259 Finally, we propose that concepts and methods with which ecologists currently define
260 stoichiometry may not be relevant at the microbial scale. Specifically, soil resource C:N:P is an
261 operational name, characterized by the total organic C and the inorganic and/or organic N and P
262 that is extractable in the soil solution. The actual pool of C (and N and P if organic N and P were
263 included) that is used in this calculation varies by extraction protocol and soil type. These pools
264 (especially C) undoubtedly contain large and variable amounts of C, N or P that is not directly
265 assimilable by soil microbes (Fig. 3). Plants may alter this assimilable pool C:N:P, both
266 indirectly and directly, by the mechanisms outlined in the paragraph in the introduction of this
267 study, including nutrient inputs, outputs and shifting plant-microbial associations. For example,
268 in a study across 9 different soil type and vegetation community combinations, microbes
269 maintained homeostasis partially through changing EEA stoichiometry, which was regulated
270 more strongly by the characteristics of the plant community than soil physiochemical variables
271 (Cui *et al.*, 2018). Shifts in plant communities within the same soil type such as we investigate in
272 this study may produce similar soil resource C:N:P between control and resource-amended

communities, but may have very different assimilable pool C:N:P, and thus may result in different microbial mechanisms used to maintain homeostasis. We encourage greater understanding of the C available to microbes from the soil resource pool. In much the same way that we measure inorganic N and P, we can dig deeper into the microbial resource C pool at a molecular scale. A number of methodological improvements already exist in the literature, including (1) isotopic tracer methods of low molecular weight (i.e. assimilable) carbon (Lynch *et al.*, 2018); (2) size-based filtration fractionation of soil extractions (Farrell *et al.*, 2011); (3) molecular-level exploration of C quality in soil extractions (i.e. with HPLC, GC/MS, nanoSIMS, NMR; Hall et al. 2011), or (4) a companion incubation of the soil extract to assess the bioavailable fraction. Any of these methods could then be used to scale the C, N or P content in the C:N:P ratio. This consideration and those suggested above should improve process-level assessment of microbial response to soil resource C:N:P across scales.

In summary, we proposed that ecosystems with strong plant community response to fertilisation would allow maintenance of microbial homeostasis with minimal microbial effort. We found little support for our hypothesis because although we found higher indications of microbial homeostasis in the ecosystem with stronger plant community response to steep resource gradients, these did not appear to be dictated by less microbial resource cost. Therefore, we remain uncertain to what extent plant community dynamics impact microbial homeostasis in ecosystems with changing plant communities across strong resource gradients. Our results highlighted issues with variability in ecosystem-level experimental systems of microbial homeostasis with a strong plant community response on the same soil type, and potential issues with how we quantify the microbial assimilable pool of soil resources. We respond with a call for further ecosystem-level investigations of microbial homeostasis where resource gradients

exist on the same soil type in natural ecosystems, such as those in long-term nutrient addition experiments. We suggest using designs that increase field-level replication, isolate potential plant-microbial associations, and enhance the molecular-level quantification of the microbial-assimilable resource pool.

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409
410 **Figure legends:**

411

412 **Figure 1.** Scatter plots of log-transformed soil available versus microbial biomass C:N (a, c) or
413 C:P (b, d), where variation in soil resources is driven by long-term N and P factorial fertilization
414 treatments in Moist Acidic Tundra (MAT: a,b) and Moist Non-acidic Tundra (MNT: c,d) at the
415 Arctic LTER at Toolik Lake, Alaska. Each point represents an individual plot in the fertilization
416 experiment and the black line indicates slope of the regression regardless of significance – a
417 significant relationship indicates non-homeostasis in the microbial resource ratio (c only).

418

419 **Figure 2.** Mean (± SE) response ratio for extracellular enzyme activity (a, d), carbon use
420 efficiency (b, e) and plant functional group abundance (c, f) for three nutrient addition treatments
421 (N, P and NP combined) relative to control plots in a long-term fertilization experiment in Moist
422 Acidic Tundra (MAT: a-c) and Moist Non-acidic Tundra (MNT: d-f) at the Arctic LTER at
423 Toolik Lake, Alaska. A significant or trending response ratio (testing difference from 0 for each
424 variable in each treatment) is indicated as + ($p < 0.1$), * ($p < 0.05$) and ** ($p < 0.01$).

425

426 **Figure 3**. Schematic of mechanisms (white background) that control pool sizes and their C:N:P
427 stoichiometry (colour background) modified from Mooshammer et al. (2014) to include the
428 relevance of plant community shifts for ecosystem-level studies, and the effect of plant
429 community studies on the soil extractable vs microbial assimilable resource pool.

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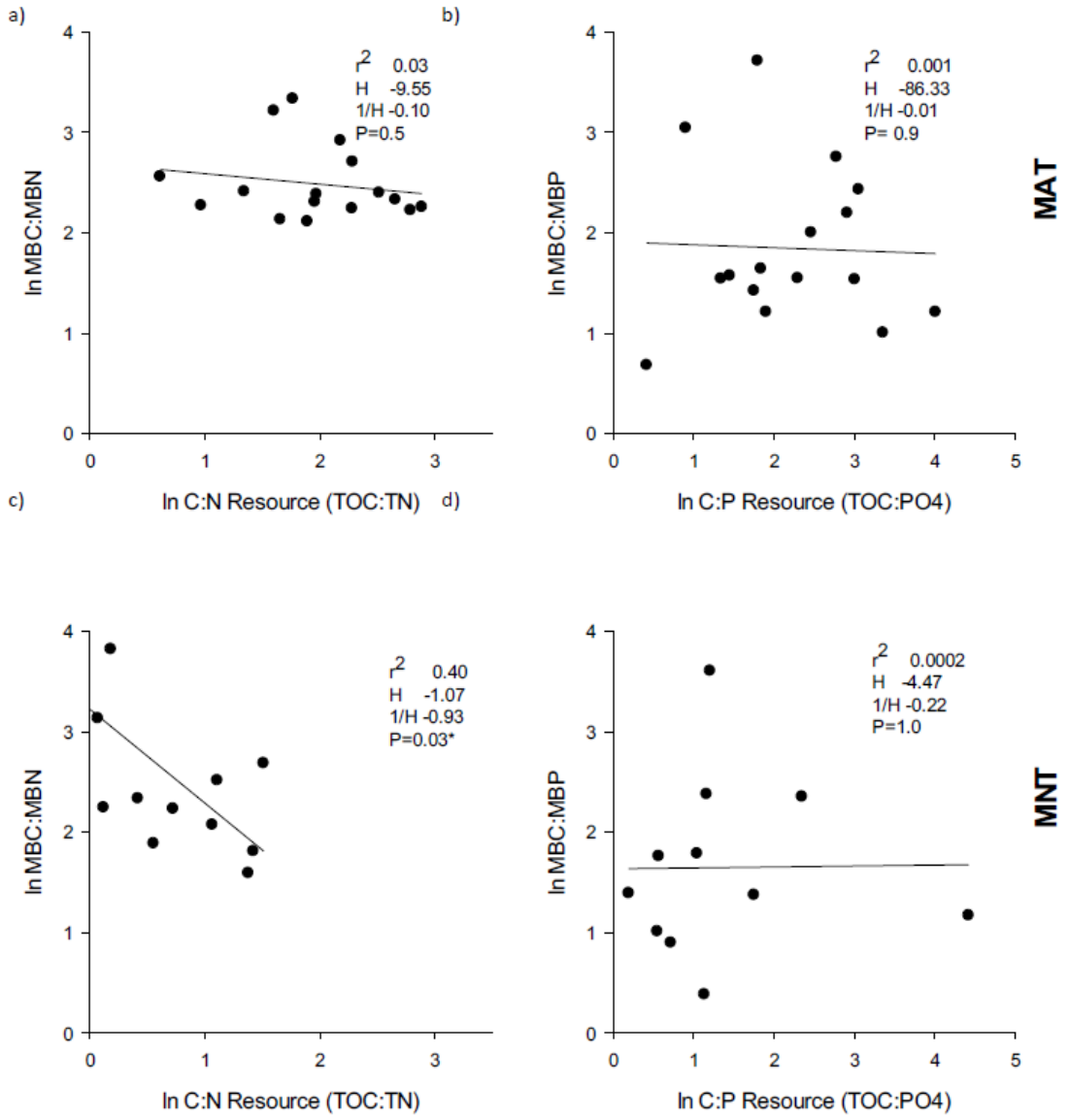
a)
b)
c)
d)
r^2 0.03
H -9.55
1/H -0.10
P=0.5
r^2 0.001
H -86.33
1/H -0.01
P= 0.9
r^2 0.40
H -1.07
1/H -0.93
P=0.03*
r^2 0.0002
H -4.47
1/H -0.22
P=1.0
ln MBC:MBN
ln MBC:MBP
ln C:N Resource (TOC:TN)
ln C:P Resource (TOC:PO4)
MAT
MNT

Figure 1.

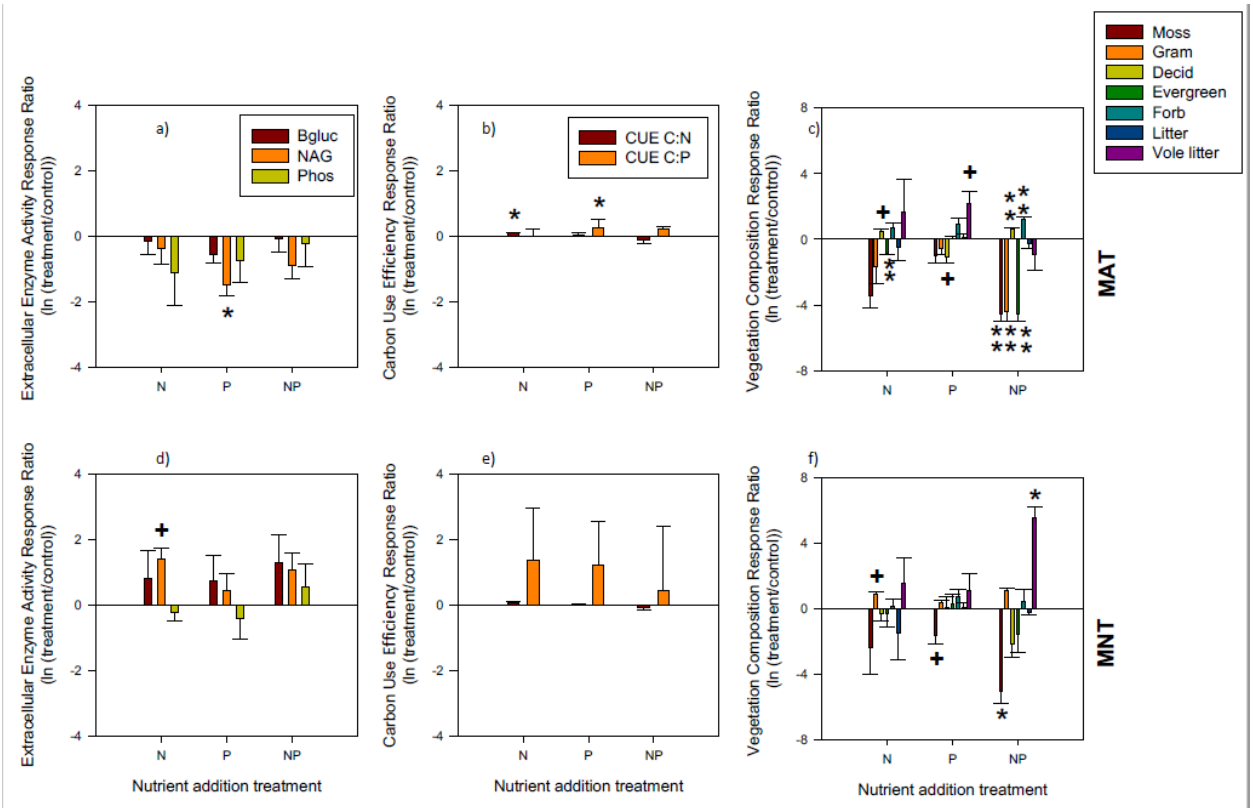
a)
b)
c)
d)
e)
f)
Bgluc
NAG
Phos
CUE C:N
CUE C:P
Moss
Gram
Decid
Evergreen
Forb
Litter
Vole litter
MAT
MNT
Extracellular Enzyme Activity Response Ratio (ln (treatment/control))
Carbon Use Efficiency Response Ratio (ln (treatment/control))
Vegetation Composition Response Ratio (ln (treatment/control))
N
P
NP
Nutrient addition treatment

Figure 2.

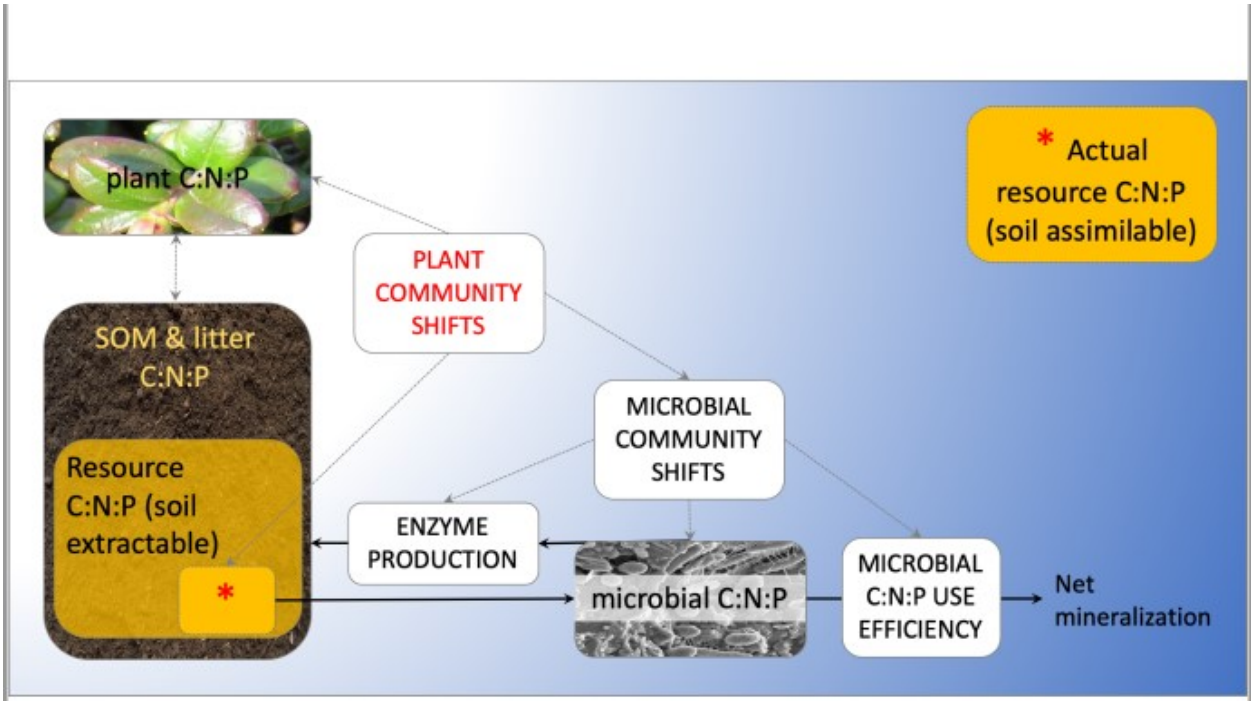
plant C:N:P
* Actual resource C:N:P (soil assimilable)
PLANT COMMUNITY SHIFTS
SOM & litter C:N:P
Resource C:N:P (soil extractable)
*
MICROBIAL COMMUNITY SHIFTS
ENZYME PRODUCTION
microbial C:N:P
MICROBIAL C:N:P USE EFFICIENCY
Net mineralization

Figure 3.