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How deep do we dig for surface soil? A comparison of patterns of microbial C:N:P stoichiometry between topsoil and subsoil along an aridity gradient

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Abstract

Microbial stoichiometry and its driving factors play crucial roles in understanding the balance of chemical elements in ecological interactions and nutrient limitations along aridity gradients. However, little is known about the variation in these features along aridity gradients due to the lack of comprehensive field investigations. Additionally, previous studies focused on the surface soil (0-10 or 0-20 cm); however, the minimum sampling depth for surface soil could impact the results of the vertical distribution of microbial stoichiometry. In the present study, we measured the variation in microbial stoichiometry, examined the major influential factors (climatic, edaphic and biotic factors) along an aridity gradient and determined whether the sampling depth affected microbial C:N:P stoichiometry. We found that the microbial C:N (topsoil: 6.59; subsoil: 6.83), C:P (topsoil: 60.2; subsoil: 60.5) and N:P ratios (topsoil: 9.29; subsoil: 8.91) varied with soil depth and that the microbial C:N ratio significantly increased with soil depth. The microbial C:N ratio significantly increased with increasing aridity for both topsoil and subsoil, while the microbial N:P ratio decreased along the aridity gradient only for the topsoil , which implied that drought-stimulated microbes tend to be more N conservative, especially for the topsoil. Among all the factors, the soil organic carbon (SOC) content and fungi to bacteria ratio exerted the largest influence on the microbial C:N, C:P and N:P ratios at both soil depths, implying that the substrate supply and microbial structure together controlled the microbial stoichiometry. The results also revealed that the aridity index (AI) and plant aboveground biomass (AGB) influenced

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the C:N ratio in microbial biomass at both soil depths, and the effects of those factors decreased in the subsoil. The results of this study suggested that the flexibility of the microbial N:P ratio should be considered when establishing the minimum sampling depth in a vertical study. The present study also represented the first attempt to examine the patterns of soil microbial stoichiometry for different soil depth along an aridity gradient.

Keywords: grassland ecosystem, C:N:P stoichiometry, soil microbial biomass, aridity gradient

1 Introduction

Ecological stoichiometry is a powerful tool for understanding the balance of chemical elements required by organisms and the functions of ecosystems (Elser et al., 2000a; Sterner and Elser, 2002). C, N and P are regarded as critical elements in global biogeochemical cycling, and C:N:P stoichiometry in soil microorganisms offers essential insight into the nutrient limitations of the organisms and communities in an ecosystem (Manzoni et al., 2010; McGroddy et al., 2004).

Few studies have addressed the pattern of microbial stoichiometry along latitudinal (Cleveland and Liptzin, 2007; Li et al., 2015; Xu et al., 2013) or environmental gradients (Li et al., 2015; Li and Chen, 2004; Li et al., 2012). For example, Cleveland and Liptzin (2007) analyzed microbial stoichiometry at the global scale and showed an increasing trend in the microbial N:P ratio with latitude. However, Li et al. (2015)

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63 summarized the data and found that the microbial N:P ratio decreased with latitude.
64 Undoubtedly, there is uncertainty in the values of microbial C:N, C:P and N:P ratios in
65 global studies, and the variations in these values might have been partially caused by
66 the numerous methods that were used in the various studies (Chen et al., 2016; Xu et
67 al., 2013). Furthermore, less exploration of soil microbial stoichiometry along an aridity
68 gradient at the regional scale impedes our ability to disentangle the trend of the changes
69 in microbial stoichiometry amid climate changes. Climate change, such as global
70 warming, is increasing the degree of aridity in drylands owing to the decreased
71 precipitation and increased evaporation (Li et al., 2017; Wang et al., 2014). Under this
72 background, key ecosystem processes that are regulated by soil microbes, such as soil
73 respiration and nutrient mineralization, may be dramatically impacted by the increased
74 degree of aridity, especially in fragile areas of arid and semiarid ecosystems (Chen et
75 al., 2014; Delgadobaquerizo et al., 2013). Therefore, we conducted a field investigation
76 across a 2100-km climatic transect in the Inner Mongolian grasslands to determine how
77 the microbial C:N, C:P and N:P ratios were affected by changing environmental
78 conditions.

79 Previous studies have also shown that a variety of abiotic factors impact microbial
80 C:N:P stoichiometry (Cleveland and Liptzin, 2007; Hartman and Richardson, 2013;
81 Manzoni et al., 2010). For instance, control experiments have found that warming can
82 indirectly affect the turnover of microbial biomass N by stimulating soil respiration
83 (Veraart et al., 2011; Butterbach-Bahl et al., 2013). Climate could exert an influence on

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84 microbial stoichiometry through changes to the microenvironment, such as soil
85 moisture and temperature, and it could also impact the availability of substrates in the
86 soil (Nielsen et al., 2009). Moreover, edaphic variables, such as SOC (Maria et al., 2014;
87 Chen et al., 2016) and soil texture (Li et al., 2015), might be associated with nutrient
88 mineralization and availability, thus influencing the C:N:P stoichiometry in microbial
89 biomass (Griffiths et al., 2012). A labeled incubation experiment showed that the
90 mineralization of organic P was mainly driven by the microbial C demands in P-poor
91 soils (Aponte et al., 2010; Heuck et al., 2015). In addition, microbial C:N, C:P and N:P
92 ratios were also affected by biotic factors such as plant productivity and the composition
93 of the microbial community (Fanin et al., 2013; Chen et al., 2016). Generally, fungi
94 exhibit a higher C:N ratio than bacteria (Michaels and Johannes, 2010); thus, a shift in
95 the fungi to bacteria ratio is expected to result in microbial stoichiometry changes (Li
96 et al., 2012; Heuck et al., 2015). However, those findings were based on literature
97 analyses or small-scale experiments, and the variations in microbial C:N, C:P and N:P
98 ratios at the regional scale have rarely been assessed systematically, and the drivers of
99 these variations need to be addressed more specifically with appropriate experimental
100 designs. Moreover, most research has focused on the top 10 cm of soil, which often has
101 high C availability and nutrient contents. It can be assumed that the effects of driving
102 factors exhibit minimal differentiation at deeper soil depths. However, soil at a deeper
103 depth might contain microbial communities that are specialized for their environment,
104 and their functions may differ from the functions of the communities in the surface soil

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105 (Ghiorse and Wilson, 1988; Zvyagintsev, 1994; Fritze et al., 2000; Blume et al., 2002).
106 Certainly, the drivers that are responsible for the variations in microbial C:N, C:P and
107 N:P ratios in deeper soil remain poorly understood. Such knowledge of the nature of
108 soil microbial stoichiometry is fundamental for understanding ecosystem function,
109 especially in soil at deeper depths, which remains highly uncertain under climate
110 change background.

111 Substrates for microorganisms, such as available nutrients and water, decline
112 exponentially with depth, and the top 20 cm of soil accumulates the greatest amount of
113 microbial biomass, thereby attracting the attention of most researchers (Fierer et al.,
114 2003; Xu et al., 2013). Soil at a 0-20 cm depth was regarded as the surface soil in some
115 studies, while other researchers divided the soil from 0-20 cm into different soil depths
116 to explore the vertical differences between these depths (Aponte et al., 2010; Peng and
117 Wang, 2016). However, most studies used 0-10 cm as the surface soil to facilitate
118 sampling and comparative research (Cleveland and Liptzin, 2007; Li and Chen, 2004;
119 Chen et al., 2016). The depth of surface soil varies among studies, and sampling depth
120 can therefore have impacts on the study of the vertical patterns in soil microbial
121 stoichiometry (Tischer et al., 2014). Given that soil represents a highly heterogeneous
122 environment, especially in terms of site-specific soil development history, it is difficult
123 to draw general conclusions (Xu et al., 2013; Camenzind et al., 2018). In addition, if a
124 large difference existed between the soil at 0-10 cm and that at 10-20 cm, microbial
125 stoichiometry would be underestimated due to the ambiguous limitation of surface soil

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(Tischer et al., 2014). To identify the soil depth that is appropriate for sampling and to improve the understanding of surface soil research at a global scale, we designed a study that divided the surface soil into 0-10 cm and 10-20 cm depths to compare the differences in microbial stoichiometry at the regional scale.

In Inner Mongolia grasslands, the aridity exhibits a gradient that increases from northeast to southwest (aridity index ranges from 0.16 to 0.54), and the vegetation types vary from meadows to typical and desert steppes, thus providing an ideal platform to better estimate the patterns and drivers of microbial C:N:P stoichiometry along an aridity gradient (Chen et al., 2014; Li et al., 2017). Moreover, we aim to compare the differences in microbial stoichiometry at different soil depths, especially in the surface soil, to identify the optimal soil depth for vertical studies on microbial stoichiometry. In particular, we tested the following hypotheses: (i) microbial C:N and C:P ratios increase and the microbial N:P ratio decreases across an aridity gradient because of differences in nutrient-use efficiency. (ii) Due to variations in resource supply among different soil depths, the effects of driving factors on microbial C:N, C:P and N:P ratios might decrease with soil depth. Finally, (iii) to adapt to the imbalance of resources, microbial C:N, C:P and N:P ratios vary between soil depths and at a depth of 10 cm, which could influence the research on the vertical patterns of microbial stoichiometry.

2 Materials and methods

2.1 Study area

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146 This study was performed across the Inner Mongolian temperate grassland, which is a
147 central part of the Eurasian steppe. The study area is located at 39.2-49.6 ℃N latitude and
148 107.8-120.1 ℃E longitude and covers an area of 440,000 km^2. From northeast to
149 southwest, the mean annual temperature increases from -1.7 to 7.7 ℃, and the mean
150 annual precipitation decreases from 402 mm to 154 mm (Table. 1). Three grassland
151 types, meadow steppe, typical steppe and desert steppe, are distributed along the
152 northeastern to southwestern gradient and are dominated by *Stipa baicalensis* and
153 *Leymus chinensis*, *S. grandis*, and *S. klemenzii*, respectively (Ma et al., 2010). The soil
154 types corresponding to the three grassland types are categorized as chernozems,
155 kastanozems, and calcisols, respectively, according to the soil classification system of
156 the Food and Agriculture Organization of the United Nations.

157 **2.2 Sampling and data collection**

158 Along this transect, a total of 58 sites that were slightly disturbed by humans and
159 domestic animals were sampled, including 10 in the meadow steppe, 28 in the typical
160 steppe, and 20 in the desert steppe (Fig. A1). At each site, three replicate soil samples
161 at depths of 0-10 cm and 10-20 cm were collected from three 1 × 1 m subplots arranged
162 diagonally in a 10 × 10 m plot. The samples were mixed to form one composite sample.
163 After gentle homogenization and removal of roots, the soil was sieved through a 2-mm
164 mesh and stored to conduct the following experiments. The total carbon (TC)
165 concentrations were measured using an elemental analyzer (Vario EL III, Elementar,
166 Germany). The soil inorganic carbon (SIC) content was determined with a carbonate

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content analyzer (Eijelkamp 08.53, Netherlands). The SOC content was calculated by subtracting the SIC from the TC. The soil elemental contents were reported in mmol kg^{-1}. Soil pH was measured in a suspension with a soil:water ratio of 1:2.5. After the removal of organic matter and carbonates, the soil texture was determined using a particle size analyzer (Malvern Masterizer 2000, UK). The AI was extracted from the CGIAR-CSI Global-Aridity and GlobalPET database (Zorner, Trabucco, Bossio, & Verchot, 2008).

2.3 Soil microbial analyses

Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were determined following the chloroform fumigation-K_2SO_4 extraction method, according to Vance et al. (1987) and Wu et al. (1990). The soil was preincubated at 25 ℃ for two weeks at a field water capacity of 40%. Then, the soil was fumigated with chloroform for 24 h in a vacuum. The fumigated and nonfumigated samples were extracted using 0.5 M K_2SO_4 with a soil:solution ratio of 1:4. The C and N contents were measured with a multi N/C analyzer (Anaytik Jena, Germany). Using a universal conversion factor of 0.45, the amounts of MBC and MBN were calculated by subtracting the amounts of extractable C and N in the nonfumigated samples from those in the fumigated samples. Microbial biomass phosphorus (MBP) was estimated according to the method described in Hedley and Stewart (1982) and modified by Wu et al. (1990). The fumigation procedure was the same as that for MBC and MBN. The fumigated and nonfumigated samples were extracted using 0.5 mol L^{-1} $NaHCO_3$ and were analyzed to

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188 determine the TP concentration using a colorimetric method. Using a universal
189 conversion factor of 0.40, the amount of MBP was calculated by subtracting the amount
190 of extractable P in the nonfumigated samples from that in the fumigated samples.
191 Phospholipid fatty acids (PLFAs) were extracted from the soil using the method
192 described by Bossio and Scow (1998). Briefly, 8 g of soil (dry weight) was used for
193 PLFA analysis. After the different phases were dried, the sample was analyzed by
194 capillary gas chromatography following transesterification for quantitative analysis
195 relative to an internal standard. The following PLFAs were used as markers for each of
196 the specific groups: for fungi, 18:1ω9c, 18:2ω6c, 18:3ω6c; for bacteria, i13:0, a13:0,
197 i14:0, i15:0, a15:0, 15:1ω6, 2OH16:0, i16:0, 16:1ω7c, 16:1ω9c, a17:0, i17:0, 17:1ω8c,
198 cy17:0, i18:0, 18:1ω7, 18:1ω5 and cy19:0.

199 **2.4 Statistical analyses**

200 The C:N, C:P and N:P ratios in the soil microbial biomass were log10 transformed
201 before analysis to improve their normality. Paired samples t-tests were used to
202 determine the differences in the soil microbial biomass C, N and P between the topsoil
203 and subsoil and the differences in the C:N:P stoichiometry ratios in the soil microbial
204 biomass. Ordinary least squares regression analyses were conducted to evaluate the
205 relationship between the C:N:P ratios in the soil microbial biomass and latitude, aridity
206 index, AGB, SOC, sand percentage and fungi to bacteria ratio (F:B ratio). The analyses
207 were performed with SPSS 19.0 software (IBM Corporation, Armonk, NY, USA). A
208 structural equation model (SEM) was used to test the multivariate effects (direct and

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indirect) on the C:N:P ratios in the microbial biomass through hypothetical factor pathways (Fig. S3). The SEM was constructed using the Amos 17.0 software package (Smallwaters Corporation, Chicago, IL, USA).

3 Results

3.1 The variation in microbial C:N:P stoichiometry between soil depths along the environmental gradient

Across all data, the soil microbial biomass C:N, C:P and N:P ratios varied by orders of magnitude and were well constrained (Fig. A2). Distinct water contents, soil bulk density, sand percentages and SOC contents were found between soil depths (P <0.05, Fig. 1a, 1b, 1c, 1f). The microbial biomass C, N and P concentrations in the topsoil were significantly larger than that in the subsoil (P <0.05, Table. 2). The C:N, C:P and N:P ratios in the microbial biomass of the topsoil were 6.59, 60.2, and 9.29, respectively, while those values in the subsoil were 6.83, 60.5 and 8.91, respectively (Table. 2). Moreover, the microbial C:N ratio in the subsoil was significantly higher than that in the topsoil (Fig. 2b).

Significant positive relationships were found between the microbial C:N ratio and latitude (Topsoil, R^2 =0.14, P< 0.01; Subsoil, R^2 =0.12, P< 0.05, Fig. 2a), while a negative relationship was found between the microbial N:P ratio and latitude (Topsoil, R^2 =0.18, P< 0.001; Fig. 2c). The results revealed a significant positive relationship between the AI and the microbial C:N ratio (Topsoil, R^2 =0.10, P< 0.05; Subsoil, R^2

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229 =0.09, P< 0.05, Fig. 2d) and significant negative relationships between the AI and the
230 microbial N:P ratio (Topsoil, R^2 = 0.10, P< 0.05; Fig. 2f). The microbial C:N ratio was
231 positively related to AGB (Topsoil, R^2 =0.06, P< 0.05, Fig. 3a), SOC (Topsoil, R^2 =0.12,
232 P< 0.01; Subsoil, R^2 =0.09, P< 0.05, Fig. 3d) and was negatively related to the sand
233 percentage (Topsoil, R^2 =0.11, P< 0.01; Subsoil, R^2 =0.11, P< 0.01, Fig. 3g). A
234 significant positive relationship was found between the microbial C:P ratio and the
235 content of soil organic matter (Subsoil, R^2 =0.08, P< 0.06, Fig. 3e). No or weak
236 associations were found between the microbial C:N, C:P and N:P ratios and the
237 aforementioned ecological factors in the subsoil (Fig. 3).

238 **3.2 Effects of driving factors on the microbial C:N:P stoichiometry at different soil**
239 **depths**

240 The final SEM adequately fit the data, as shown by several robust goodness-of-fit
241 measures (P value and minimum discrepancy). The model explained 38% (topsoil) and
242 27% (subsoil) of the variation in the microbial C:N ratio, 17% and 19% of that in the
243 microbial C:P ratio, and 29% and 16% of that in the microbial N:P ratio (Fig. 4a, b, c,
244 d). Effects of AI, AGB, SOC and the F:B ratio on the microbial C:N ratio were found
245 at both soil depths (Fig. 4a, 4b). The SOC made the largest positive contribution to the
246 variation in the microbial C:N ratio in the topsoil (Fig. 4a, 4b). We found direct effects
247 of the sand percentage, SOC and F:B ratio on the microbial C:P ratio at both soil depths,
248 and the SOC content made the largest contribution to the variation in the microbial C:P
249 ratio in the topsoil, which was higher than that in the subsoil (Fig. 4e, 4f). Influences of

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250 sand% and the SOC content on the microbial N:P ratio were found in the topsoil, while
251 the F:B ratio and the SOC explained most of the variation in the microbial N:P ratio in
252 the subsoil (Fig. 4e, 4f).

253 **4 Discussion**

254 **4.1 The pattern of microbial C:N, C:P and N:P ratios along latitude and the**
255 **aridity gradient**

256 The microbial C:N ratio demonstrated a significant increase with increasing latitude, in
257 contrast to the decrease that was demonstrated for the microbial N:P ratio. Such results
258 paralleled the results of studies on ecological stoichiometry, which revealed that the
259 C:N ratio of microorganisms increased with latitude, while the N:P ratio decreased with
260 latitude, suggesting increasing N limitations in microorganism ecosystems in high-
261 latitude areas (Li et al., 2015; Chen et al., 2016). The regional-scale microbial
262 stoichiometry followed the global-scale stoichiometry patterns that were observed for
263 plant leaves (Reich and Oleksyn, 2004; Yuan et al., 2011), litter (McGroddy et al.,
264 2004), and soil (Sardans et al., 2012), conforming to the substrate age hypothesis, which
265 predicts young soils to be N-limited, whereas old soils tend to be P limited (Walker and
266 Syers, 1976; Vitousek et al., 2010). As stated in our hypothesis, we observed that the
267 microbial C:N ratio significantly increased with increasing aridity, while the microbial
268 N:P ratio decreased along the aridity gradient, indicating that increasing aridity affects
269 ecological stoichiometry by mediating the growth rate of microorganisms in semiarid

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regions (Elser et al., 2000b; Peng and Wang, 2016). Dan and Wang noted that increasing aridity reduced the soil microbial abundance in drylands, and a decreased growth rate in dry areas might result in decreased allocation to P-rich ribosomal RNA (and thus higher C:P and N:P ratios) (Wang et al., 2014; Maestre et al., 2015). Additionally, drought decreased the C:N ratio in microbial biomass, which serves as a protective mechanism as microbes decrease their nitrogen use efficiency (NUE, the ratio of N invested in growth over total N uptake) and tend to be more N conservative under dry climatic conditions (Mooshammer et al., 2014; Delgado-Baquerizo et al., 2017). Moreover, under dry climate conditions, the soil microbial communities shift from r-strategists (fast-growing copiotrophs) to K-strategists (slow-growing oligotrophs), as microorganisms with K-strategies have lower nutrient demands (N and P) and growth rates, invest more nutrients into extracellular enzymes to gain limited nutrients and thus have higher cellular C:N:P ratios than r-strategists (Fierer et al., 2007; Fierer et al., 2010). Our study further illustrated the latitudinal pattern of microbial stoichiometry and first attempted to examine the variation in microbial stoichiometry along an aridity gradient at the regional scale.

4.2 Direct effects of ecological factors on controlling microbial C:N, C:P and N:P ratios at different soil depths

Among the ecological factors examined, our study found that the patterns of microbial C:N and C:P ratios were associated with SOC and the F:B ratio, suggesting that the available C and microbial community structure together regulated the shift in microbial

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291 stoichiometry. If the environmental parameters were considered individually, SOC was
292 found to be significantly positively related to the microbial C:N and C:P ratios, which
293 is consistent with the results observed from global data analysis, suggesting that SOC
294 may control microbial stoichiometry by mediating the substrate stoichiometry, e.g., the
295 soil C:N and C:P ratios (Hartman and Richardson, 2013; Maria et al., 2014;
296 Mooshammer et al., 2014). In deeper soil, microbial metabolic processes are limited by
297 C availability and energy (C), such as denitrification and P mineralization (Fierer et al.,
298 2003; Peng and Wang, 2016; Camenzind et al., 2018). SEM also illustrated that the
299 microbial community structure is an important feature in determining microbial
300 stoichiometry. The F:B ratio has recently been found to have a vital influence on the
301 patterns of microbial C:N and N:P ratios in soil at a large scale (Chen et al., 2016). An
302 experiment indicated that fungi have lower resource requirements and higher C:N and
303 C:P ratios than bacteria, and thus microbial C:N:P stoichiometry impacted the microbial
304 community structure as a result of the F:B ratio (Mouginot et al., 2014). In our study,
305 the lower F:B ratio might have led to a shift in the microbial nutrient stoichiometry at
306 deeper soil depths (Tischer et al., 2014). Overall, the SEM highlighted the important
307 role of the C availability and microbial community structure in driving the variations in
308 microbial C:N, C:P and N:P ratios at both soil depths.

309 Moreover, AGB and AI also exerted direct influences on microbial C:N or C:P ratios,
310 and those impacts mainly acted in the topsoil but were weaker in the subsoil, supporting
311 our hypothesis. The climate imposes important controls on both the plant community

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and the microbial taxa along with their interactions with soil nutrients (Chadwick et al. 1999; Vitousek 2004; Oleksyn 2004). At the same time, the meadow steppe ecosystem with high productivity maintained relatively high soil C and N contents, which resulted in high C:P and N:P ratios in these regions; thus, plant productivity exerted a positive influence on microbial C:N (Aponte et al., 2010; Manzoni et al., 2010). Because of the vertical distribution of those influences, the effects might decrease with soil depth.

Interestingly, our results revealed that the microbial N:P ratio was mainly impacted by the F:B ratio and SOC, while the sand percentage and SOC had direct negative effects on the ratio in the subsoil, suggesting the flexibility of microbial stoichiometry in response to distinct resource supplies between soil depths (Peng and Wang, 2016). The soil depth affected the microbial biomass N and P, which decreased nearly twofold from the topsoil to the subsoil (Table 2). However, the results showed that the N and P cycles responded asymmetrically to soil depth, which might be attributed to the high variability in P availability (Li et al., 2015; Zechmeister-Boltenstern et al., 2016). Generally, P is mostly derived from parent material, while N is mainly a biological element (Vitousek and Farrington, 1997; Vitousek et al., 2010). Therefore, it is believed that P variations regulate large-scale patterns in microbial N:P stoichiometry and nutrient-use strategies (Camenzind et al., 2018; Heuck et al., 2015). With a high proportion of sand, the soil becomes porous, which may lead to increased leaching of available P to deeper soil depths (Otten et al., 1999; Achbergerov áand Nah álka, 2011). Similarly, P leaching caused by weathering led to a shift in the N:P ratio in the soil, and

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a vertical study found a high variation in the N:P ratio between soil depths across a large scale. The high spatial heterogeneity of the N:P ratio in soil and soil microbial biomass therefore indicates that the N:P ratio could be an indicator of the ecosystem nutrient status at deeper soil depths.

4.3 How deep should we dig to evaluate the microbial stoichiometry in surface soil?

The results showed significant differences in the sand percentage, SOC content and F:B ratio between soil depths, suggesting that the resource supplies between topsoil and subsoil were distinct. We also observed that the microbial C:N, C:P and N:P ratios varied between soil depths, indicating the flexibility of the microbial community in response to distinct resource supplies between soil depths (Tian et al., 2010; Peng and Wang, 2016). Similar findings were found in the top 16 cm of soil in a Mediterranean oak forest (0-8 cm and 8-16 cm), where the microbial nutrient ratios (C:N, C:P and N:P) varied with soil depth (Aponte et al., 2010). Tischer et al. (2014) sampled the top 20 cm of soil (0-5 cm, 5-10 cm, 10-20 cm) and observed that the microbial C:N ratio changed with soil depth. Moreover, sampling to a depth of 10 cm showed a significant difference in the microbial N:P ratio (Tischer et al., 2014). The detection of the differences in the microbial N:P ratio in our study depended strongly on the sampling depth, suggesting that the microbial N:P ratio might provide insight into the nature of ecosystem nutrient limitations in a vertical study (Cleveland and Liptzin, 2007; Fierer et al., 2010). In addition, SEM also showed that the microbial N:P ratio was controlled

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by multiple driving factors at different soil depths, indicating that a 0-10 cm or shallower sampling interval should be used when studying the vertical patterns in the microbial N:P ratio.

5 Uncertainties and perspectives

The first uncertainty was related to the determination of fungal and bacterial biomasses by PLFA markers, which have limited targets for fungi and bacteria. This uncertainty should be noted when interpreting the results in the present study. Methodological advances in sequencing approaches might be used to more accurately index the microbial community and reveal insights into the regulation of microbial C:N:P stoichiometry in distinct soil microbial taxa or functional groups. Second, the theory used to construct the model is another source of uncertainty; the theory related to the drivers of microbial stoichiometry used in this study was mostly derived from a literature review and summarized data. In future research, more control experiments with the manipulation of C availability, especially at deeper soil depths, would further improve our understanding of the changes in microbial stoichiometry and nutrient limitations under the impacts of global change.

6 Conclusion

The ratios of C, N, and P in the microbial biomass were 6.59:60.2:9.29 in the topsoil, which deviated from the 6.83:60.5:8.91 ratio in the subsoil. Moreover, significant difference was found in the microbial C:N ratio between topsoil and subsoil, indicating

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that the flexibility of microbial stoichiometry should be considered for vertical study. In addition, drought decreased the C:N ratio in microbial biomass, consistent with the perspective that microbes mediate their nitrogen use efficiency and tend to be more N conservative under drier climatic conditions. The microbial N:P ratio trend along the aridity gradient was consistent with the growth rate hypothesis that a decreased growth rate in dry areas results in decreased allocation to P-rich ribosomal RNA and thus a higher N:P ratio. These findings confirmed the importance of SOC, the microbial structure and soil texture in shaping the pattern of microbial stoichiometry in semiarid grassland systems. The influence of ecological factors decreased from topsoil to subsoil, as well as the decline in climatic, edaphic and biotic factors. Overall, these results illustrated N and P limitation in microbial biomass at deeper soil depths along aridity gradient and limited responses to ecological factors in the subsoil.

Author contributions. HH. WM and YY devised the study. YL carried out the experiment and data analyses. DK, YC and DC assisted with the data analyses and interpretation. XN, TW, XZ, MZ and HB assisted with the experiment. All authors contributed to the preparation of the paper.

Competing interests. The authors declare that they have no conflict of interest.

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396 **Table 1.** Basic information of study sites

Biome	Latitude (°N)	Longitude (°E)	MAP (mm)	MAT (°C)	Aridity Index	AGB (g ·cm^{-3})	Dominant species
MS	48.1(43.9–49.6)	119(116-120)	353(262–381)	–0.45(–1.81–1.71)	0.48 (0.38-0.54)	136(88-168)	*Stipa baicalensis* *Leymus chinensis*
TS	45.6(43.5-49.5)	117(114-119)	304 (205–402)	1.11(-2.09-3.29)	0.37 (0.25–0.50)	102(49.4-159.8)	*Stipa grandis* *Stipa kryovii*
DS	41.7(39.2-43.6)	115(108-113)	223(154–293)	5.63(4.13-7.67)	0.23 (0.16-0.32)	43.4(24.6-76.5)	*Stipa klemenzii* *Stipa breviflora*

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398 Note: Data represent the means, with minimum and maximum values in parentheses. MS, meadow steppe; TS, typical steppe; DS, desert steppe.

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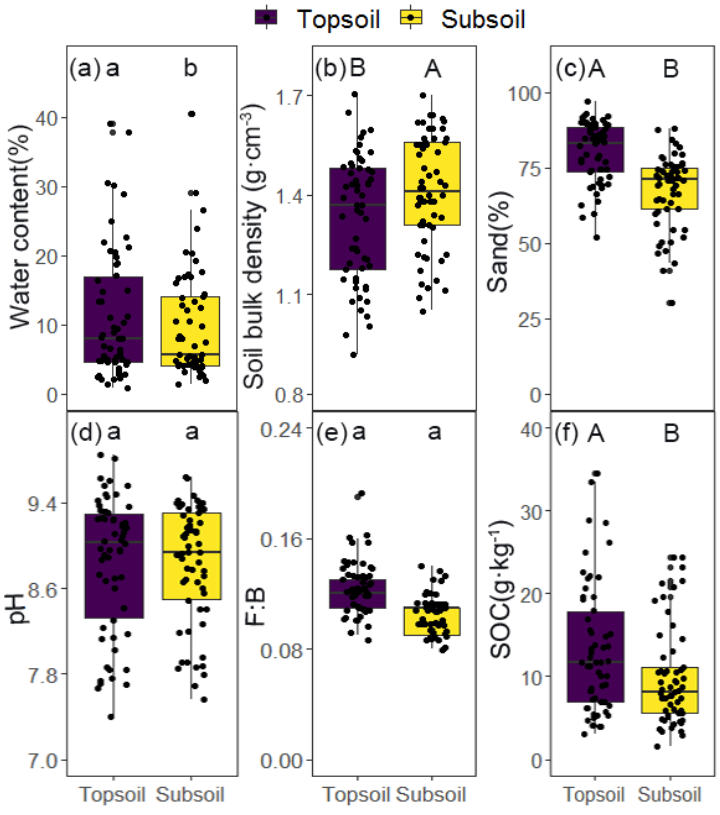

Figure 1. Basic characteristics of study sites across the inner Mongolia grassland at different soil depths. Different letters indicate significant differences between soil depths on log10-transformed data (paired t-test, lower letter, $P<0.05$; upper case letter, $P<0.001$). MS, meadow steppe; TS, typical steppe; DS, desert steppe.

404 **Table 2.** The microbial biomass C, N and P concentrations and microbial C:N:P stoichiometric ratios across the Inner Mongolian grassland at

405

Biome	MBC (mmol ·kg^{-1})	MBN (mmol ·kg^{-1})	MBP (mmol ·kg^{-1})	Microbial biomass C:N	C:P	N:P
0-10 cm	21.8(18.5-25.1)A	3.23(2.80-3.67)A	0.38(0.32-0.44)A	6.59(6.26-6.91)a	60.2(55.6-64.8)a	9.29(8.0-9.97)a
10-20 cm	14.5(12.4-16.6)B	2.08(1.81-2.35)B	0.24(0.21-0.27)B	6.83(6.50-7.15)b	60.5(56.0-65.1)a	8.91(8.35-9.49)a

406

407 Note¨ Different letters indicate significant differences between soil depths based on log10-transformed data (paired t-test, lower-case letters,

408 P<0.05; upper-case letters, P<0.001). MS, meadow steppe; TS, typical steppe; DS, desert steppe.

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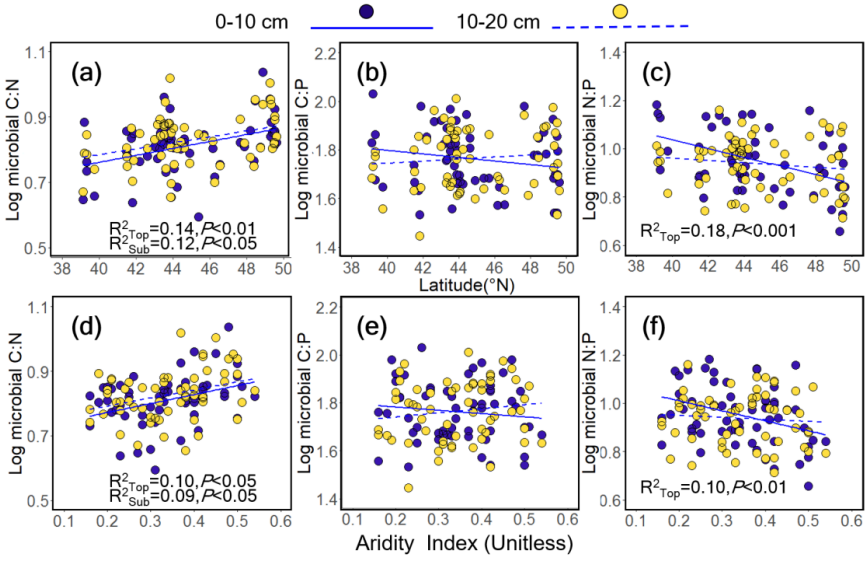

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411 **Figure 2.** Relationships between the C:N, C:P and N:P ratios in soil microbial

412 biomass and latitude (a-c) and aridity index (d-f) in the Inner Mongolian grassland.

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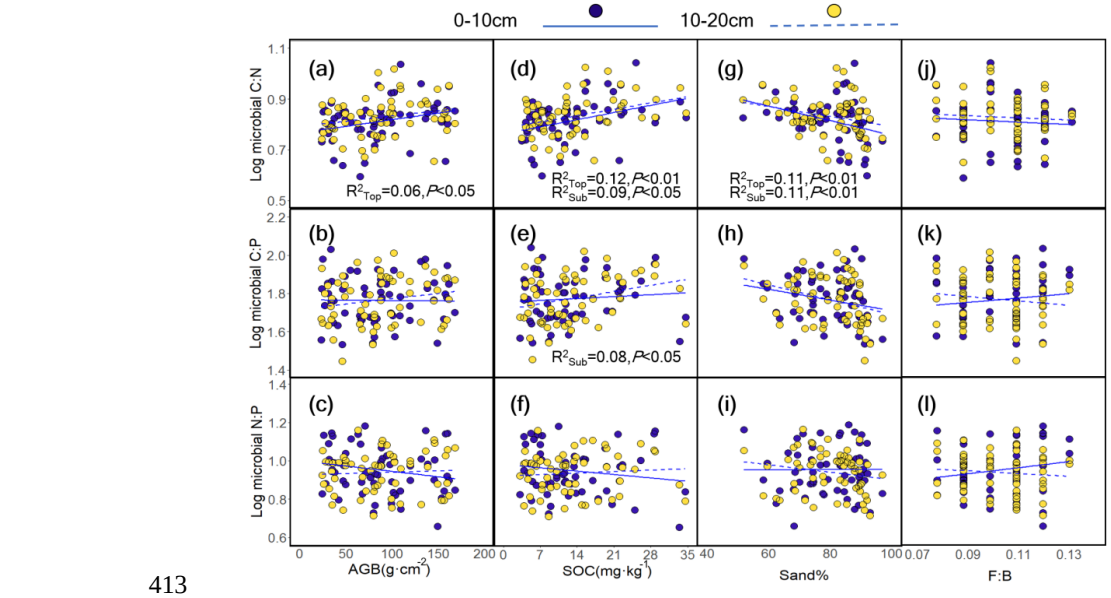

Figure 3. Relationships between the C:N, C:P and N:P ratios in the soil microbial biomass and AGB (a-c), SOC (d-f), sand percentage (g-i) and F:B ratio (j-l). AGB, above ground biomass; SOC, soil organic carbon; F:B ratio, fungi to bacteria ratio.

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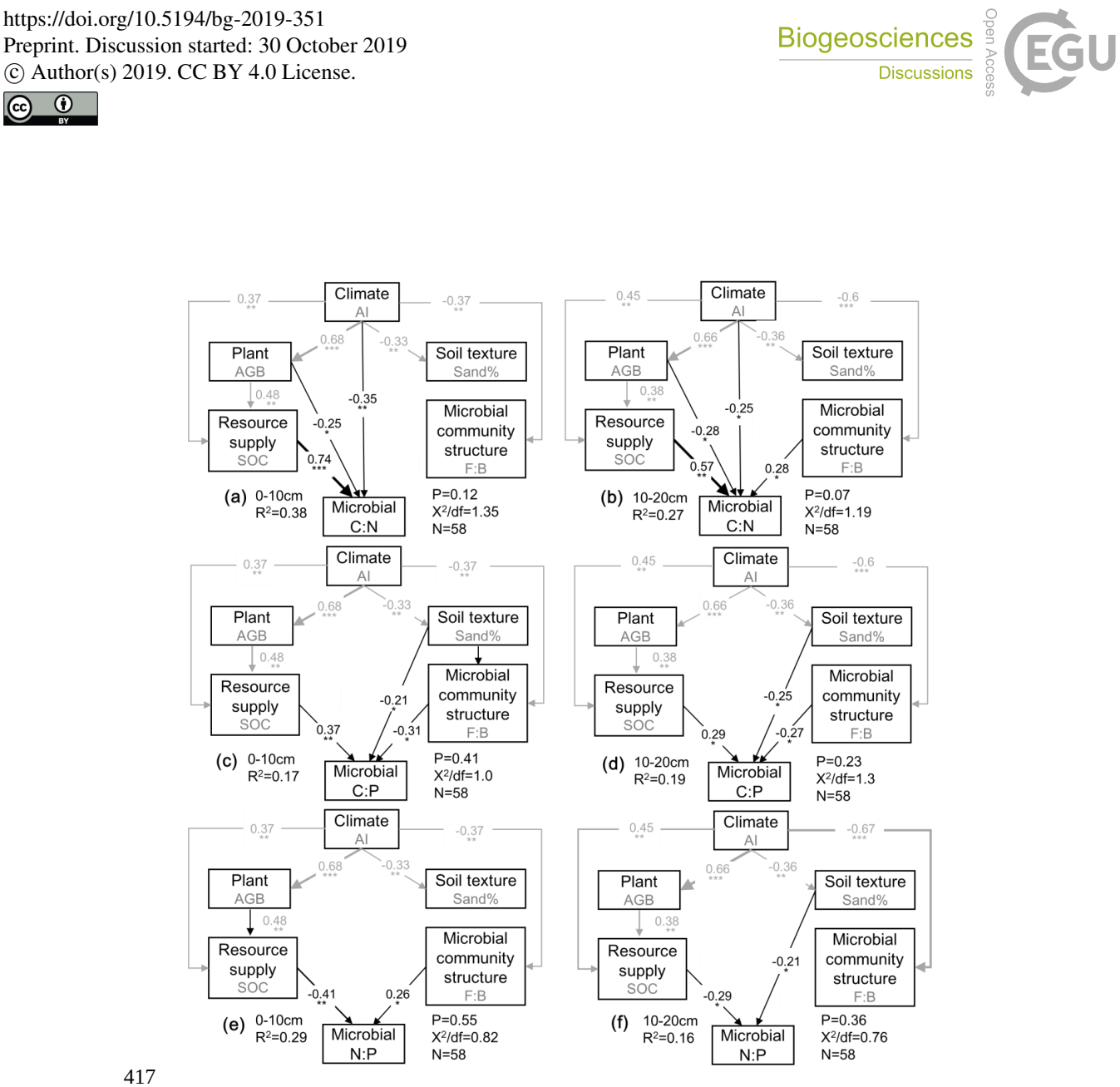

Figure 4. The structural equation model (SEM) shows the direct and indirect influences of various ecological factors on the microbial C:N (a,b), C:P (c,d) and N:P (e-f) ratios in the topsoil and subsoil. Black and gray arrows indicate direct and indirect pathways, respectively. Numbers on the arrows indicate standardized path coefficients, proportional to the arrow width. R^2 indicates the variation of the microbial C:N and C:P ratios explained by the model. *, P<0.05; **, P<0.01; ***, P<0.001.

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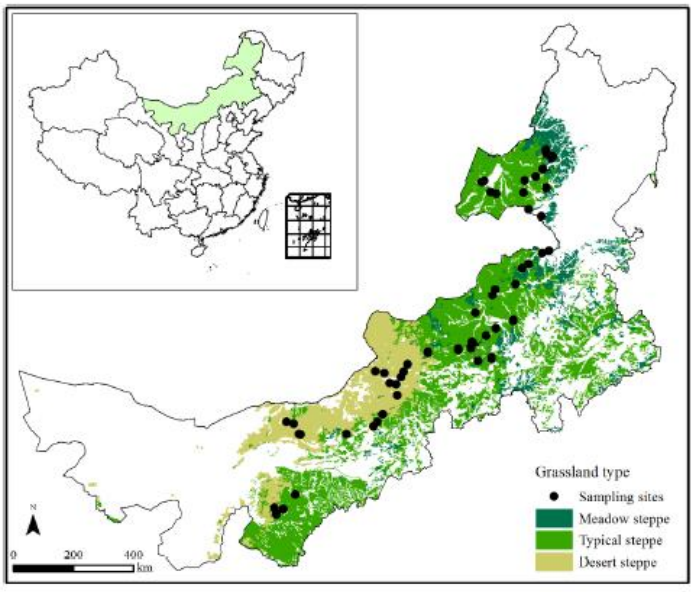
424 **Appendix**



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426 **Figure A1.** Geographic locations of the sampling sites in the Inner Mongolian grassland

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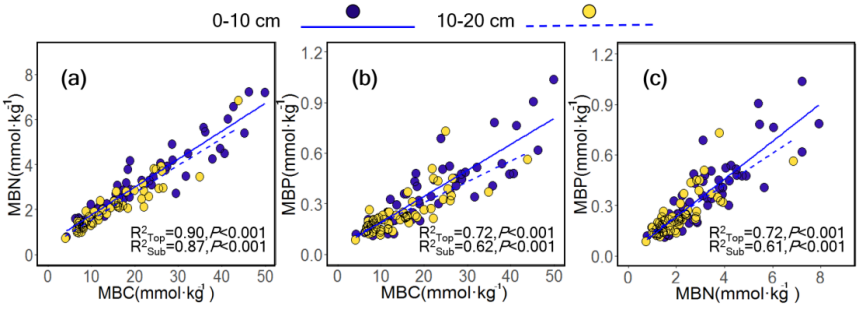

Figure A2. Relationships between the soil microbial biomass C, N and P concentrations

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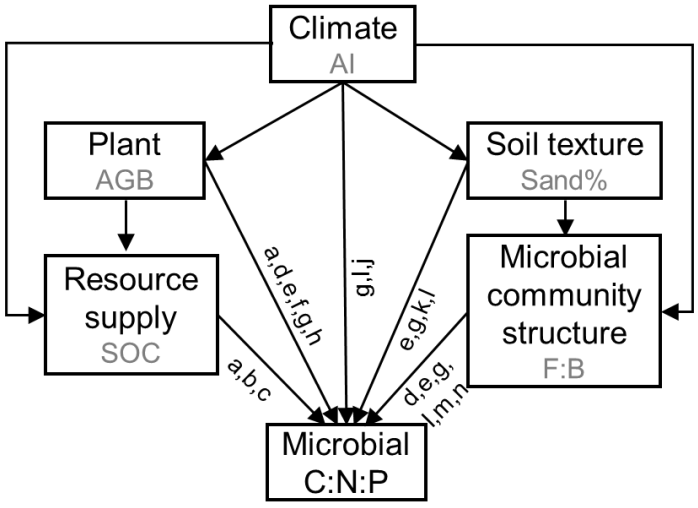

429

430 **Figure A3.** Hypothetical model showing how ecological factors affect microbial C:N:P

431 stoichiometry

432 **Table A1.** The references to support hypothetical models

Number	Reference
a	Maria, M., Wolfgang, W., Sophie, Z. B., and Andreas, R.: Stoichiometric imbalances between terrestrial decomposer communities and their resources: mechanisms and implications of microbial adaptations to their resources, Frontiers in Microbiology, 5, 22, 2014.
b	Hartman, W. H., and Richardson, C. J.: Differential Nutrient Limitation of Soil Microbial Biomass and Metabolic Quotients (q CO2): Is There a Biological Stoichiometry of Soil Microbes?, 8, e57127, 2013.
c	Mooshammer, M., Wanek, W., Hämmerle, I., Fuchslueger, L., Hofhansl, F., Knoltsch, A., Schnecker, J., Takriti, M., Watzka, M., Wild, B., Keiblinger, K. M., Zechmeister-Boltenstern, S., and Richter, A.: Adjustment of microbial nitrogen use efficiency to carbon:nitrogen imbalances regulates soil nitrogen cycling, Nature Communications, 5, 3694, 10.1038/ncomms4694
d	Aponte, C., Marañón, T., García, L. V., Johnson, D., Vile, M., and Wieder, K.: Microbial C, N and P in soils of Mediterranean oak forests: influence of season, canopy cover and soil depth, Biogeochemistry, 101, 77-92, 2010.
e	Cleveland, C. C., and Liptzin, D.: C:N:P Stoichiometry in Soil: Is There a "Redfield Ratio" for the Microbial Biomass?, Biogeochemistry, 85, 235-252, 2007.
f	Manzoni, S., Trofymow, J. A., Jackson, R. B., and Porporato, A.: Stoichiometric controls on carbon, nitrogen, and phosphorus dynamics in decomposing litter, Ecological Monographs, 80, 89-106, 2010.

https://doi.org/10.5194/bg-2019-351
Preprint. Discussion started: 30 October 2019

g Zechmeister-Boltenstern, S., Keiblinger, K. M., Mooshammer, M., Peñuelas, J., Richter, A., Sardans, J., and Wanek, W.: The application of ecological stoichiometry to plant–microbial–soil organic matter transformations, Ecological Monographs, 85, 133-155, 2016.

h Li, Y., Wu, J., Liu, S., Shen, J., Huang, D., Su, Y., Wei, W., and Syers, J. K.: Is the C:N:P stoichiometry in soil and soil microbial biomass related to the landscape and land use in southern subtropical China?, Global Biogeochemical Cycles, 26, -, 2012.

i Chen, Y., Kou, D., Li, F., Ding, J., Yang, G., Fang, K., and Yang, Y.: Linkage of plant and abiotic properties to the abundance and activity of N-cycling microbial communities in Tibetan permafrost-affected regions, Plant and Soil, 10.1007/s11104-018-3845-9, 2018.

j Wang, C., Wang, X., Liu, D., Wu, H., Lü, X., Fang, Y., Cheng, W., Luo, W., Jiang, P., Shi, J., Yin, H., Zhou, J., Han, X., and Bai, E.: Aridity threshold in controlling ecosystem nitrogen cycling in arid and semi-arid grasslands, Nature Communications, 5, 4799, 10.1038/ncomms5799

k Li, P., Yang, Y., Han, W., and Fang, J.: Global patterns of soil microbial nitrogen and phosphorus stoichiometry in forest ecosystems, Global Ecology & Biogeography, 23, 979-987, 2015.

l Xu, X., Thornton, P. E., and Post, W. M.: A global analysis of soil microbial biomass carbon, nitrogen and phosphorus in terrestrial ecosystems, Global Ecology & Biogeography, 22, 737–749, 2013.

m Ross, D. J., and Tate, K. R.: Microbial C and N in litter and soil of a southern beech (Nothofagus) forest: Comparison of measurement procedures, Soil Biology and Biochemistry, 25, 467-475

n Tischer, A., Potthast, K., and Hamer, U.: Land-use and soil depth affect resource and microbial stoichiometry in a tropical mountain rainforest region of southern Ecuador, Oecologia, 175, 375-393, 2014.

433

https://doi.org/10.5194/bg-2019-351
Preprint. Discussion started: 30 October 2019

434 **References:**

435 Achbergerová, L. and Nahálka, J.: Polyphosphate - an ancient energy source and active
436 metabolic regulator, Microbial Cell Factories, 10, 63, 2011.
437 Aponte, C., Marañón, T., García, L. V., Johnson, D., Vile, M. and Wieder, K.:
438 Microbial C, N and P in soils of Mediterranean oak forests: influence of season,
439 canopy cover and soil depth, Biogeochemistry, 101, 77-92, 2010.
440 Camenzind, T., Hättenschwiler, S., Treseder, K. K., Lehmann, A. and Rillig, M. C.:
441 Nutrient limitation of soil microbial processes in tropical forests, 88, 4-21, 2018.
442 Chen, D., Mi, J., Chu, P., Cheng, J., Zhang, L., Pan, Q., Xie, Y. and Bai, Y.: Patterns
443 and drivers of soil microbial communities along a precipitation gradient on the
444 Mongolian Plateau, Landscape Ecology, 30, 1669-1682, 2014.
445 Chen, Y. L., Chen, L. Y., Peng, Y. F., Ding, J. Z., Li, F., Yang, G. B., Kou, D., Liu, L.,
446 Fang, K. and Zhang, B. B.: Linking microbial C:N:P stoichiometry to microbial
447 community and abiotic factors along a 3500 - km grassland transect on the
448 Tibetan Plateau, Global Ecology & Biogeography, 25, 1416-1427, 2016.
449 Cleveland, C. C. and Liptzin, D.: C:N:P Stoichiometry in Soil: Is There a "Redfield
450 Ratio" for the Microbial Biomass?, Biogeochemistry, 85, 235-252, 2007.
451 Delgado-Baquerizo, M., Powell, J. R., Hamonts, K., Reith, F., Mele, P., Brown, M. V.,
452 Dennis, P. G., Ferrari, B. C., Fitzgerald, A. and Young, A.: Circular linkages
453 between soil biodiversity, fertility and plant productivity are limited to topsoil
454 at the continental scale, New Phytologist, 215, 2017.
455 Delgadobaquerizo, M., Maestre, F. T., Gallardo, A., Bowker, M. A., Wallenstein, M.
456 D., Quero, J. L., Ochoa, V., Gozalo, B., García-gómez, M. and Soliveres, S.:
457 Decoupling of soil nutrient cycles as a function of aridity in global drylands,
458 Nature, 502, 672, 2013.

https://doi.org/10.5194/bg-2019-351
Preprint. Discussion started: 30 October 2019

459 Elser, J. J., Fagan, W. F., Denno, R. F., Dobberfuhl, D. R., Folarin, A., Huberty, A.,
460 Interlandi, S., Kilham, S. S., Mccauley, E. and Schulz, K. L.: Nutritional
461 constraints in terrestrial and freshwater food webs, Nature, 408, 578-580, 2000a.
462 Elser, J. J., Sterner, R. W., Gorokhova, E., Fagan, W. F., Markow, T. A., Cotner, J. B.,
463 Harrison, J. F., Hobbie, S. E., Odell, G. M. and Weider, L. W.: Biological
464 stoichiometry from genes to ecosystems, 3, 540-550, 2000b.
465 Fierer, N., Bradford, M. A. and Jackson, R. B.: Toward an ecological classification of
466 soil bacteria, Ecology, 88, 1354-1364, 2007.
467 Fierer, N., Schimel, J. P. and Holden, P. A.: Variations in microbial community
468 composition through two soil depth profiles, Soil Biology & Biochemistry, 35,
469 167-176, 2003.
470 Fierer, N., Strickland, M. S., Liptzin, D., Bradford, M. A. and Cleveland, C. C.: Global
471 patterns in belowground communities, Ecology Letters, 12, 1238-1249, 2010.
472 Griffiths, B. S., Spilles, A. and Bonkowski, M.: C:N:P stoichiometry and nutrient
473 limitation of the soil microbial biomass in a grazed grassland site under
474 experimental P limitation or excess, Ecological Processes, 1, 6, 2012.
475 Hartman, W. H. and Richardson, C. J.: Differential Nutrient Limitation of Soil
476 Microbial Biomass and Metabolic Quotients (q CO 2): Is There a Biological
477 Stoichiometry of Soil Microbes?, 8, e57127, 2013.
478 Heuck, C., Weig, A. and Spohn, M.: Soil microbial biomass C:N:P stoichiometry and
479 microbial use of organic phosphorus, Soil Biology and Biochemistry, 85, 119-
480 129, 2015.
481 Li, H., Zhang, J., Hu, H., Chen, L., Zhu, Y., Shen, H. and Fang, J.: Shift in soil microbial
482 communities with shrub encroachment in Inner Mongolia grasslands, China,
483 European Journal of Soil Biology, 79, 40-47, 2017.

https://doi.org/10.5194/bg-2019-351
Preprint. Discussion started: 30 October 2019

484 Li, P., Yang, Y., Han, W. and Fang, J.: Global patterns of soil microbial nitrogen and
485 phosphorus stoichiometry in forest ecosystems, Global Ecology &
486 Biogeography, 23, 979-987, 2015.
487 Li, X. Z. and Chen, Z. Z.: Soil microbial biomass C and N along a climatic transect in
488 the Mongolian steppe, Biology & Fertility of Soils, 39, 344-351, 2004.
489 Li, Y., Wu, J., Liu, S., Shen, J., Huang, D., Su, Y., Wei, W. and Syers, J. K.: Is the
490 C:N:P stoichiometry in soil and soil microbial biomass related to the landscape
491 and land use in southern subtropical China?, Global Biogeochemical Cycles, 26,
492 2012.
493 Maestre, F. T., Delgado-Baquerizo, M., Jeffries, T. C., Eldridge, D. J., Ochoa, V.,
494 Gozalo, B., Quero, J. L., Garc á-G ómez, M., Gallardo, A., Ulrich, W., Bowker,
495 M. A., Arredondo, T., Barraza-Zepeda, C., Bran, D., Florentino, A., Gait án, J.,
496 Guti érrez, J. R., Huber-Sannwald, E., Jankju, M., Mau, R. L., Miriti, M., Naseri,
497 K., Ospina, A., Stavi, I., Wang, D., Woods, N. N., Yuan, X., Zaady, E. and
498 Singh, B. K.: Increasing aridity reduces soil microbial diversity and abundance
499 in global drylands, Proceedings of the National Academy of Sciences, 112,
500 15684, 2015.
501 Manzoni, S., Trofymow, J. A., Jackson, R. B. and Porporato, A.: Stoichiometric
502 controls on carbon, nitrogen, and phosphorus dynamics in decomposing litter,
503 Ecological Monographs, 80, 89-106, 2010.
504 Maria, M., Wolfgang, W., Sophie, Z. B. and Andreas, R.: Stoichiometric imbalances
505 between terrestrial decomposer communities and their resources: mechanisms
506 and implications of microbial adaptations to their resources, Frontiers in
507 Microbiology, 5, 22, 2014.
508 McGroddy, M. E., Daufresne, T. and Hedin, L. O.: Scaling of C : N : P stoichiometry

https://doi.org/10.5194/bg-2019-351
Preprint. Discussion started: 30 October 2019

509 in forests worldwide: Implications of terrestrial redfield-type ratios., Ecology,
510 85, 2390-2401, 2004.
511 Mooshammer, M., Wanek, W., Hämmerle, I., Fuchslueger, L., Hofhansl, F., Knoltsch,
512 A., Schnecker, J., Takriti, M., Watzka, M., Wild, B., Keiblinger, K. M.,
513 Zechmeister-Boltenstern, S. and Richter, A.: Adjustment of microbial nitrogen
514 use efficiency to carbon:nitrogen imbalances regulates soil nitrogen cycling,
515 Nature Communications, 5, 3694, 2014.
516 Otten, W., Gilligan, C. A., Watts, C. W., Dexter, A. R. and Hall, D.: Continuity of air-
517 filled pores and invasion thresholds for a soil-borne fungal plant pathogen,
518 Rhizoctonia solani, Soil Biology and Biochemistry, 31, 1803-1810, 1999.
519 Peng, X. and Wang, W.: Stoichiometry of soil extracellular enzyme activity along a
520 climatic transect in temperate grasslands of northern China, Soil Biology &
521 Biochemistry, 98, 74-84, 2016.
522 Reich, P. B. and Oleksyn, J.: Global patterns of plant leaf N and P in relation to
523 temperature and latitude, Proceedings of the National Academy of Sciences of
524 the United States of America, 101, 11001, 2004.
525 Sardans, J., Rivas-Ubach, A. and Peñuelas, J.: The elemental stoichiometry of aquatic
526 and terrestrial ecosystems and its relationships with organismic lifestyle and
527 ecosystem structure and function: a review and perspectives, Biogeochemistry,
528 111, 1-39, 2012.
529 Sterner, R. W. and Elser, J. J., Ecological Stoichiometry: The Biology of Elements
530 From Molecules to The Biosphere, 2002.
531 Tian, H. Q., Chen, G. S., Zhang, C., Melillo, J. M. and Hall, C. A. S.: Pattern and
532 variation of C:N:P ratios in China's soils: a synthesis of observational data,
533 Biogeochemistry, 98, 139-151, 2010.

https://doi.org/10.5194/bg-2019-351
Preprint. Discussion started: 30 October 2019

534 Tischer, A., Potthast, K. and Hamer, U.: Land-use and soil depth affect resource and
535 microbial stoichiometry in a tropical mountain rainforest region of southern
536 Ecuador, Oecologia, 175, 375-393, 2014.
537 Vance, E. D., Brookes, P. C. and Jenkinson, D. S.: An extraction method for measuring
538 soil microbial biomass C, Soil Biology and Biochemistry, 19, 703-707, 1987.s
539 Vitousek, P. M. and Farrington, H. J. B.: Nutrient limitation and soil development:
540 Experimental test of a biogeochemical theory, 37, 63-75, 1997.
541 Vitousek, P. M., Porder, S., Houlton, B. Z. and Chadwick, O. A.: Terrestrial phosphorus
542 limitation: mechanisms, implications, and nitrogen-phosphorus interactions,
543 Ecological Applications, 20, 5-15, 2010.
544 Walker, T. W. and Syers, J. K.: The fate of phosphorus during pedogenesis, Geoderma,
545 15, 1-19, 1976.
546 Wang, C., Wang, X., Liu, D., Wu, H., Lü, X., Fang, Y., Cheng, W., Luo, W., Jiang, P.,
547 Shi, J., Yin, H., Zhou, J., Han, X. and Bai, E.: Aridity threshold in controlling
548 ecosystem nitrogen cycling in arid and semi-arid grasslands, Nature
549 Communications, 5, 4799, 2014.
550 Wu, J., Joergensen, R. G., Pommerening, B., Chaussod, R. and Brookes, P. C.:
551 Measurement of soil microbial biomass C by fumigation-extraction—an
552 automated procedure, Soil Biology & Biochemistry, 22, 1167-1169, 1990.
553 Xu, X., Thornton, P. E. and Post, W. M.: A global analysis of soil microbial biomass
554 carbon, nitrogen and phosphorus in terrestrial ecosystems, Global Ecology &
555 Biogeography, 22, 737–749, 2013.
556 Yuan, Z. Y., Chen, H. Y. H. and Reich, P. B.: Global-scale latitudinal patterns of plant
557 fine-root nitrogen and phosphorus, Nature Communications, 2, 344, 2011.

https://doi.org/10.5194/bg-2019-351
Preprint. Discussion started: 30 October 2019

558 Zechmeister-Boltenstern, S., Keiblinger, K. M., Mooshammer, M., Peñuelas, J., Richter,
559 A., Sardans, J. and Wanek, W.: The application of ecological stoichiometry to
560 plant–microbial–soil organic matter transformations, Ecological Monographs,
561 85, 133-155, 2016.

562