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1 **Stronger microbial nutrient limitations in subsoil along the precipitation gradient of**

2 **agroecosystem: Insights from soil enzyme activity and stoichiometry**

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21   **Abstract:** Soil extracellular enzymes are central in terrestrial ecosystem responses to climate

22   change, and their research can be crucial for assessing microbial nutrient demand. However,

23   the effects of climate-induced precipitation patterns on soil microbial nutrient demand in

24   different soil profiles of agroecosystems are rarely studied. Here, we present how the

25   precipitation gradient affects soil enzymes related to carbon (C), nitrogen (N) and phosphorus

26   (P) cycling and identified microbial nutrient limitation determinants at five depth intervals (0-

27   10, 10-20, 20-30, 30-40 and 40-50 cm) in seven agroecosystems. We found that N- and P-

28   acquiring enzymes have a tendency to increase or decrease, but C- acquiring enzymes did not

29   change along the precipitation gradient throughout soil profiles. Soil pH and moisture were the

30   most important factors affecting the enzyme activity in 0-50 cm. Our results also revealed a

31   crucial soil boundary (at 20 cm) that differentiated responses of microbial nutrient limitation

32   to precipitation changes. In the topsoil (0-20 cm), the stoichiometry of soil nutrients did not

33   vary with precipitation. Microbial P limitation was exacerbated with increased precipitation,

34   which was controlled by soil pH and moisture in the topsoil. In contrast, in the subsoil (20-50

35   cm), soil nutrient stoichiometry decreased with increasing precipitation, and microbial C and

36   P limitation displayed a positive correlation with precipitation. Furthermore, microbial P

37   limitation tended to be stronger in the subsoil than in the topsoil along the precipitation gradient.

38   Microbial C and P limitation was regulated by the soil nutrients and their stoichiometry in the

39   subsoil. Our study is an essential step in soil enzyme activity and stoichiometry response to

40   precipitation in agroecosystems and provides novel insights into understanding microbial

41   nutrient limitation mechanisms in soil profiles along the precipitation gradient.

43 Northeast China Transect
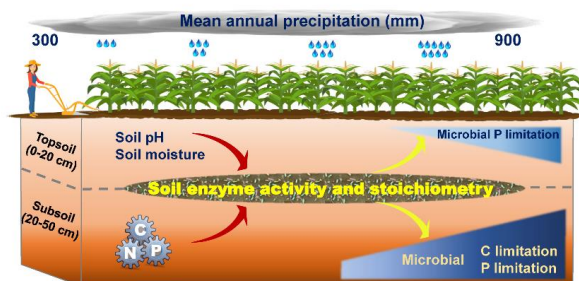
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45 **Graphical abstract** Soil enzyme activity and stoichiometry along a precipitation gradient

46 (300-900 mm) among soil profiles in agricultural ecosystems. Microbial C limitation,

47 microbial carbon limitation; Microbial P limitation, microbial phosphorus limitation.

48 **1. Introduction**

49   Changes in precipitation can lead to shifts in belowground ecological processes that are

50 interlinked with primary production, damaging regional crop production in agroecosystems

51 (Franco et al., 2019; Lesk et al., 2016; Sünnemann et al., 2021). Under rapid precipitation

52 changes, carbon (C), nitrogen (N), and phosphorus (P) cycles may become uncoupled because

53 of the different degrees of control exerted on the supply of these elements by biological and

54 geochemical processes (Delgado-Baquerizo et al., 2013). To meet the food demand, farmers

55 are continuously raising N and P inputs, altering C availability, and leading to a further

56 imbalance in the stoichiometry of microbial substrates in soils (Peñuelas et al., 2013; Schleuss

57 et al., 2021). A combination of precipitation and agricultural activities more drastically alters

58 the patterns, magnitude, and extent of nutrient limitation in soil (Marklein and Houlton, 2012).

59 Such limitation is directly relevant to the fate of soil microbial processes (Camenzind et al.,

60 2018), but the extent and nature of microbial nutrient limitation has never been systematically

61   assessed in agroecosystems along a precipitation gradient. Thus, the underlying ecological

62   mechanisms governing microbial nutrient limitation in agroecosystems are required to support

63   future predictions of precipitation changes.

64   Extracellular enzyme activities (EEA) play a central role in regulating imbalances

65   between resources and growth demands in microbe self-organizational processes (Sinsabaugh

66   and Follstad Shah, 2011; Sinsabaugh et al., 2015). Extracellular enzymes are mediators of

67   decomposition and mineralization of N and P (Moorhead and Sinsabaugh, 2006; Schimel and

68   Weintraub, 2003), and are also indicators of microbial nutrient demand (Moorhead et al., 2016).

69   According to resource allocation theory, when a resource is scarce, microorganisms

70   preferentially invest metabolic resources into extracellular enzyme production to mine the

71   limited element (Allison and Vitousek, 2005; Johnson et al., 2010; Mooshammer et al., 2014).

72   Due to the context dependence of enzyme activity, EEA has gradually received greater

73   attention and become a popular method for examining the direct effects of precipitation on the

74   functional potential of the soil microbial community in field experiments (Henry, 2013;

75   Weedon et al., 2011).

76   The theory of enzyme stoichiometry further reflects the balance between microbial

77   nutrient demand and soil nutrient resource supplies, highlighting the limiting factors of

78   biogeochemical cycling in terrestrial ecosystems (Sinsabaugh et al., 2009; Xu et al., 2017;

79   Zechmeister-Boltenstern et al., 2015). Sinsabaugh et al. (2008) developed an approach to

80   visualize the relative C, N, and P controls on soil microbial communities by plotting the ratios

81   of activities for enzymes associated with C, N and P acquisition. Moorhead et al. (2016)

82   proposed that vector lengths and angles can be used to improve the quantification and

83   visualization of these controls. A global meta-analysis exhibited that enzymatic C:P and N:P

84   ratios were inversely related to precipitation, while the ratio of C:N acquisition increased with

85   precipitation (Sinsabaugh et al., 2008). In forest ecosystems, the C:N, C:P, and N:P acquisition

86   ratios were negatively related to precipitation from a depth of 0-10 cm soil (Xu et al., 2017).

87   However, Cui et al. (2019a) found a shift from P to N limitation of microorganisms with an

88   increase in precipitation from 300 mm to 900 mm in grassland ecosystems from the 0-20 cm

89   soil layer. Therefore, generalizing the effects of precipitation on soil EEA can be challenging

90   and elusive, and it is necessary to strengthen our research on EEA patterns at regional scales.

91   To the best of our knowledge, there remains a lack of studies on the impact of precipitation

92   gradients on soil enzyme in agroecosystems, especially in deeper soil profiles.

93   The enzyme distribution in the soil matrix reflects the dynamic interactions among the

94   soil structure, environmental conditions, and microbial nutrient limitation (Allison et al., 2007).

95   Microbial nutrient limitation has been studied in soil profiles (Brockett et al., 2012; Jing et al.,

96   2017; Peng and Wang, 2016; Zuo et al., 2018). Changes in abiotic environments due to long-

97   term climate drivers, such as alterations in soil moisture (Morugán-Coronado et al., 2019), soil

98   pH (Kivlin and Treseder, 2014; Rousk et al., 2010), soil C:N ratio (Zhou et al., 2020) and soil

99   type (Wakelin et al., 2008), can affect microbial nutrient limitation in complex and

100   unpredictable ways (Burns et al., 2013; Li et al., 2018; Xu et al., 2020a). These changes often

101   occur in strong gradients with depth (Dove et al., 2020; Jia et al., 2019; Stone et al., 2014).

102   Especially in agroecosystems, cropland soils have separate soil layers due to different

103   intensities of human disturbance: topsoil (0-20 cm) and subsoil (below 20 cm) (Yan et al.,

104   2017). It is therefore not surprising that most soil properties (e.g., moisture and nutrients) and

105   microbial communities generally vary with soil depth (Kanal and Kõlli, 1996; Li et al., 2014).

106   More importantly, emerging evidence shows that subsoil may exhibit a stronger response to

107   climate change in microbial activities and functioning (Fontaine et al., 2007) in association

108   with microbial nutrient limitation. However, it remains unclear whether the relationships

109   between microbial nutrient limitation and precipitation in topsoil and subsoil differ at the

110   regional scale. Identifying the mechanisms that govern these divergent responses of microbial

111   nutrient limitation to precipitation in soil profiles is critical to minimize uncertainty in future

112   soil health and function.

113   In this study, we focused on soil enzyme activity in five soil layers of seven maize fields

114   along a steep precipitation gradient. We examined the effect of precipitation on microbial

115   nutrient limitation concerning the drivers in agroecosystem soil profiles. We developed the

116   following hypotheses: 1) microbial nutrient limitation may exhibit more significant responses

117   to precipitation in the subsoil than in the topsoil; and 2) the driving factors change with an

118   increase in depth. This study determines the microbial nutrient limitation patterns of semi-arid

119   to humid regions and identifies drivers of variations in these patterns across soil profiles in

120   agroecosystems.

121   **2. Materials and methods**

122   *2.1. Study site and experiment design*

123   The study was conducted in seven agroecosystems in the Northeast China Transect

124   (NECT), a climatic zone with significant precipitation gradients and a critical component of

125   the International Geosphere-Biosphere Programme terrestrial transects (Ni and Zhang, 2000;

126   Zhang et al., 1997). At latitudes ranging from 42° N to 44° N and longitudes from 123° E to

127   128° E within the NECT, precipitation and aridity demonstrate steep gradients (Ni and Zhang,

128   2000). From west to east, the MAP increases from 381 mm to 840 mm, and the mean annual

129   temperature (MAT) declines from 6.2 °C to 4.1 °C. We standardized our site selection by

130   focusing on maize planting areas. These agricultural sites, which have existed for over 50 years,

131   are located in flat areas and are managed conventionally under the same government and

132   according to the same policies. Conventional tillage included fall plowing, spring cultivation,

133   planting. The depth of agricultural tillage (commonly referred to as plough sole) is about 20

134   cm (Yan et al., 2017). Fertilizer for maize was applied with 200 kg N ha^{-1}, 70 kg P ha^{-1} and

135   90 kg K ha^{-1} as the base fertilizer at planting. The weeds were controlled using broad-spectrum

136   herbicides and supplemented as required with manual hoeing (Guo et al., 2020). The soil types

137   are classified as salt-alkali, light chernozem, chernozem, black soil, and dark brown soil, based

138   on the Chinese soil classification system (Xiong and Li, 1987). The basic properties of the

139   study sites are listed in Table S1.

140   *2.2. Soil sampling and characterization*

141   The seven sites, Baihe (BH), Longwan (LW), Dongliao (DL), Changchun (CC), Nongan

142   (NOA), Sijianfang (SJF) and Changling (CL), are positioned along a precipitation gradient.

143   Three plots (approximately 50×50 m) situated 50-100 m from each other were randomly

144   selected at each site. After removing the litter and rocks, the soil was sampled by horizon to a

145   maximum depth of 50 cm. Samples were taken discretely at five depth intervals (0-10 cm, 10-

146   20 cm, 20-30 cm, 30-40 cm and 40-50 cm). Three cores of soil, each with a diameter of 5.5 cm,

147   were randomly collected and gently mixed to form one composite sample for each layer of

148   each plot. In total, 105 soil samples (7 sites $\times$ 5 layers $\times$ 3 plots) were collected during the

149   maize harvest period in September 2018 to minimize the effects of agricultural management.

150   Each sample was separated into two portions. The first fresh soil samples were

151   immediately placed inside coolers containing ice packets for transport, returned to the

152   laboratory, and stored at 4 °C. SM was determined gravimetrically using 10 g of fresh soil

153   samples dried at 105 °C for 48 h to a constant weight. The second portion was air-dried for

154   edaphic property analyses. The soil pH was measured in a soil-water suspension (1:5 soil to

155   water ratio) using a glass electrode with a pH meter (Thermo Fisher Scientific Inc., USA).

156   Total C (TC) and N (TN) contents were determined using a Vario MICRO cube Elemental

157   Analyzer. Total P was first digested using H_2SO_4-$HClO_4$, and then the total P (TP)

158   concentration was measured using a continuous flow analyzer.

159   *2.3. Soil enzyme activity*

160   The potential activities of five hydrolytic enzymes, including two C-acquiring enzymes,

161   β-glucosidase (BG) and β-1,4-cellobiosidase (CB), two N-acquiring enzymes, β-N-

162   acetylglucosaminidase (NAG) and Leucine aminopeptidase (LAP), one P-acquiring enzyme,

163   acid phosphatase (AP), were measured at a temperature of 25 °C based on a modified version

164   of the methods described by Saiya-Cork et al. (2002) and German et al. (2011). The mass of

165   1.5 g of fresh soil was accurately recorded and 125 ml of 50 mM acetate buffer was added and

166   the samples were homogenized to prepare a suspension. The methodology of enzymes is

167   described in detail Fig. S1. The pre-experimental results exhibited that the activity was the

168   highest in the dark after 4 h of incubation at 25 °C. Fluorescence plates were read at 365 nm

169   excitation and 450 nm emission using a microplate reader (BioTek Synergy HTX, Winooski,

170   Vermont, USA). Eight analytical replicates were conducted per sample. Enzymatic activity

171   was enumerated as nanomoles of substrate released per gram of dry soil and incubation time

172   (nmol h^{-1} g^{-1}).

173   *2.4. Quantification of microbial nutrient limitation*

174   Resource limitation was measured by vector analysis of enzymatic stoichiometry (length

175   and angle) (Moorhead et al., 2013) according to the relative activities of enzymes that acquire

176   C, N and P. The rationale for using the relative activities of C-, N-, and P-acquiring enzymes

177   to interpret the relative limitation of C, N, and P for microorganisms is based on ecosystem

178   stoichiometry and metabolic theory (Allison et al., 2010). Relatively longer vector lengths

179   express a greater C limitation, while vector angles $< 45°$ and $> 45°$ indicate the relative degrees

180   of N and P limitation, respectively (Moorhead et al., 2016). The vector length (unitless) and

181   angle (°) are calculated as follows:

182   Vector length = $SQRT((lnBG/ln(NAG+LAP))^2 + (lnBG/lnAP)^2)$

183   Vector angle = Degrees (ATAN2((lnBG/lnAP), (lnBG/ln (NAG+LAP))))

184   *2.5. Statistical analysis*

185   The Shapiro-Wilk normality test was performed using the R version 3.5.3 'stats' package

186   (R Development Core Team, 2019). We examined the relationships between precipitation and

187   different soil properties, enzyme activities, and C:N, C:P, and N:P ratios using linear

188   regressions. C-acquiring enzymes were calculated as BG + CB, N-acquiring enzymes were

189   calculated as NAG + LAP, and P-acquiring enzyme was represented by AP. Redundancy

190   analysis (RDA) was performed to analyze the relationships between soil enzyme activities and

191   selected soil properties for 105 samples by the packages "vegan" (Oksanen et al., 2019),

192   "adespatial" (Dray et al., 2018). We used the variation inflation factor (VIF) ("vif.cca" function)

193   to assess multicollinearity among soil properties (VIF < 10). The significance of RDA

194   correlations was tested Monte Carlo permutation test.

195   Similarly, we explored the relationships between precipitation and vector length and angle

196   for each soil profile. The analysis of covariance (ANCOVA) was used to detect the differences

197   in the slope of regression lines between the angle and length of vector and precipitation among

198   soil profiles by SPSS software version 26.0 (IBM Corporation, Armonk, New York, USA).

199   The angle and length of vector were dependent variables, precipitation was covariates, and soil

200   profiles were a grouping factor with five levels. A significant soil profiles × covariate

201   interaction would indicate that the slope of the precipitation–vector relationship differs among

202   soil profiles. When the interaction was significant, the homogeneity of slopes assumption of

203   ANCOVA was rejected (Poorter et al., 2008). Differences at the $P < 0.05$ level were considered

204   statistically significant. To determine the relative influences of environmental variables on the

205   ecoenzymatic vectors in the five soil layers, we used the rdacca.hp function to calculate the

206   independent adjusted R-squared (R^2) and the percentage of each explanatory variable in the

207   RDA, running 999 permutations in the 'rdacca.hp' R package (Lai and Peres-neto, 2020). This

208   function performs variation partitioning and hierarchical partitioning to calculate the unique,

209   shared, and independent contributions of each predictor to the explained variation (R^2 and

210   adjusted R^2) in canonical analysis (RDA) by applying the hierarchy algorithm of Chevan and

211   Sutherland (1991).

212   **3. Results**

213   *3.1. Soil properties and their stoichiometry*

214   The physicochemical properties and soil nutrient ratios displayed various patterns in

215 topsoil (0-20 cm) and subsoil (20-50 cm) along the precipitation gradient (Fig. 1). The soil pH

216 decreased significantly from 9.36 to 4.46 with increasing precipitation. In contrast, soil

217 moisture and nutrients (TC, TN, and TP) were positively correlated with precipitation, but this

218 strong correlation was not observed in the subsoil for TC. Interestingly, for these components

219 of soil nutrient stoichiometry components (TC:TN, TC:TP, and TN:TP), significant positive

220 relationships with precipitation were observed in the subsoil but not in the topsoil.
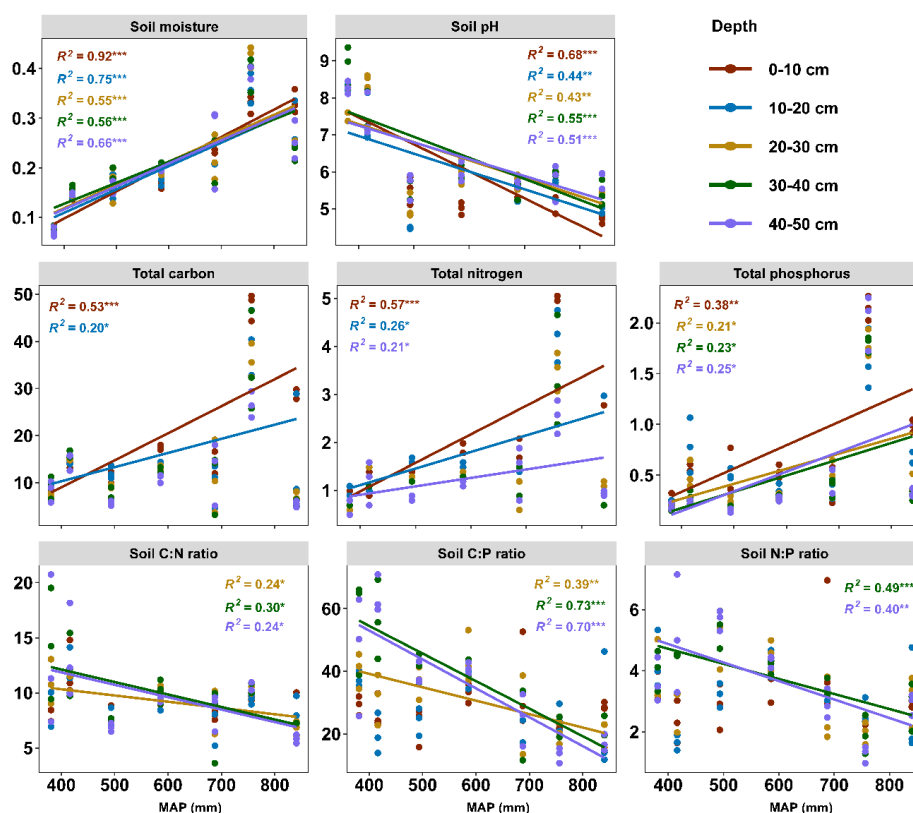

221

222 **Figure 1.** Linear regression between soil properties and MAP for each soil layer. Colored lines

223 represent the regression of each soil layer (n=105). Statistics (R^2 and P values) for the

224 regression are indicated using the corresponding colored text: *** $P < 0.001$, **$P < 0.01$, and

225   *$P < 0.05$. Only significant differences are shown.

226   *3.2. Soil enzyme activity and stoichiometry across precipitation gradient*

227   The soil enzyme patterns were dissimilar to soil nutrient patterns along the precipitation

228   gradient (Fig. 2). C-acquiring enzyme activities were unaffected by precipitation. The N-

229   acquiring enzyme activities decreased across the precipitation gradient in all soil profiles, while

230   P-acquiring enzyme activities were positively correlated with precipitation at a depth of 0-30

231   cm but not at that of 30-50 cm. The C:P enzyme stoichiometry pattern was the opposite of that

232   of the P-acquiring enzyme. With increasing precipitation, the C:N and N:P enzyme nutrient

233   stoichiometries demonstrated increasing and decreasing trends, respectively; however, both

234   stoichiometries responded to precipitation more drastically in the subsoil than in the topsoil.

235   The major environmental variables shaping the soil enzyme pattern were determined through

236   RDA (Fig. 3). According to the RDA results, approximately 33.58% of the variance in total

237   soil enzyme activity can be explained by environmental variables. The first two RDA axes

238   explained 30.04% of the relationships between enzymes and the environment (24.79% and

239   5.25% for the first and second axes, respectively). According to the model selection results,

240   soil pH and moisture were the most important factors affecting the enzyme activity.

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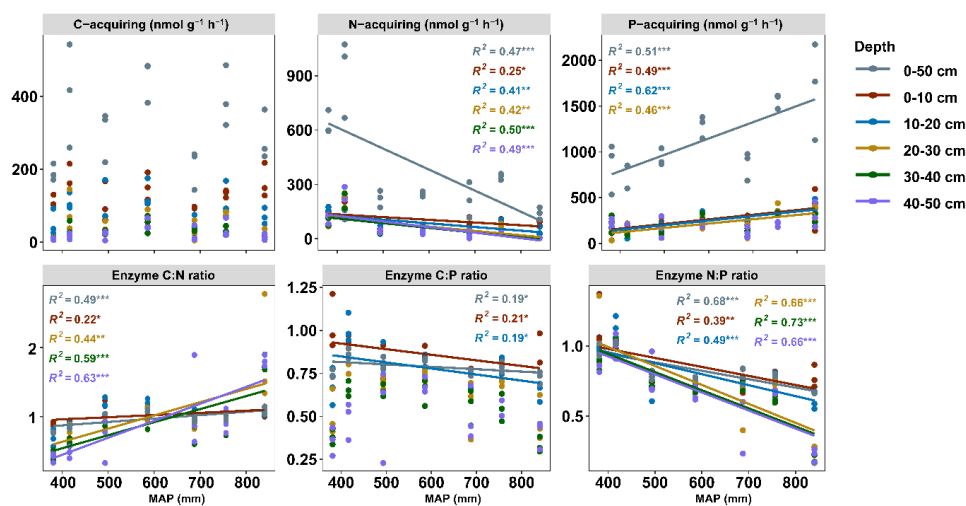
Figure 2. Patterns of enzyme activity and stoichiometry along the precipitation gradient for each soil layer. Colored lines represent the regression of each soil layer (n=105). Statistics (R^2 and P values) for the regression are indicated using the corresponding colored text: *** $P <$ 0.001, **$P < 0.01$, and *$P < 0.05$. Only significant differences are shown. C, N, and P represent carbon, nitrogen, and phosphorus.

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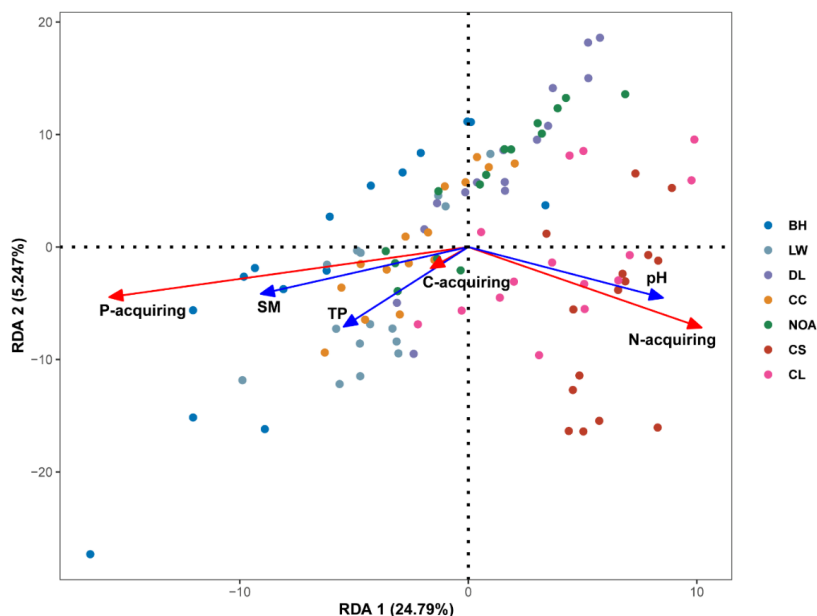
Figure 3. Redundancy analysis of 105 soil samples based on soil enzyme activity and dominant

environmental factor ordination biplot. The overall model is significant ($P < 0.001$).

Environmental factors: SM, soil moisture; pH, soil acidity and alkalinity; TP, total phosphorus.

3.3. Soil microbe metabolic limitation and its driving factors

The nutrient limitation of soil microbes was calculated using enzyme stoichiometry and

quantified by vector angle and length (Fig. 4). Vector angles ranging from 36.1° to 80.5° were

positively related to the precipitation, and they displayed a more intense correlation with

precipitation in the subsoil than in the topsoil (Fig. 4a; Table S2). We also subdivided the

driving factors for nutrient limitation according to the soil layer (Fig. 5). We observed that pH

had the largest independent effects on vector angle in topsoil, explaining 69.41% and 44.78%

of the variance in the depth ranges of 0-10 cm and 10-20 cm, respectively (Fig. 5a). From the

topsoil to the subsoil, the soil physicochemical properties (soil pH and moisture) variance

260   explanation ranged from 85.64% to 16.79% (Fig. 5a). In contrast, the interpretations of soil

261   nutrients and their stoichiometry ranged from 14.36% to 83.21% (Fig. 5a). Vector length was

262   positively correlated with precipitation only in the subsoil (Fig. 4b). The interpretation of soil

263   nutrient stoichiometry with respect to the vector length grew stronger with depth (Fig. 5b).
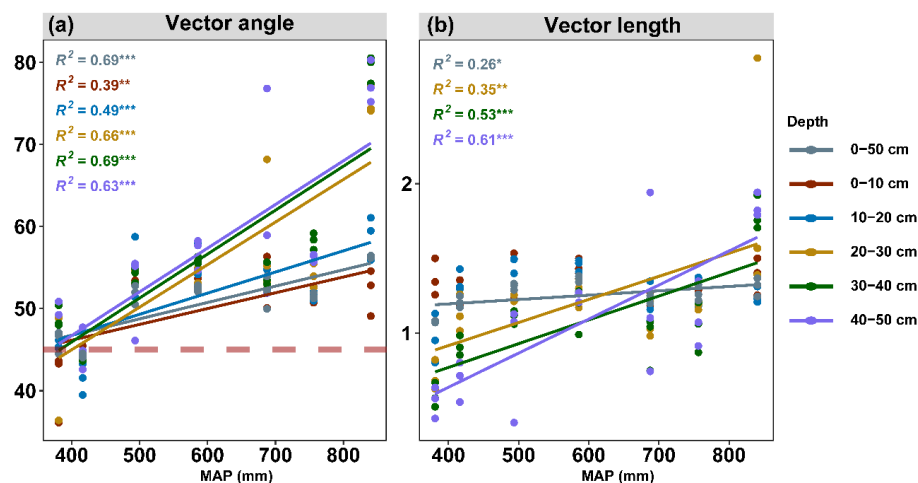
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265   **Figure 4.** Effects of MAP (mm) on microbial N/P limitation (A) and microbial C limitation

266   (B). Soil microbial N/P limitation is represented by vector angles: > 45° represents P limitation,

267   and < 45° represents N limitation. Soil microbial C limitation is represented by vector lengths,

268   where microbial C limitation increases as the values increase.

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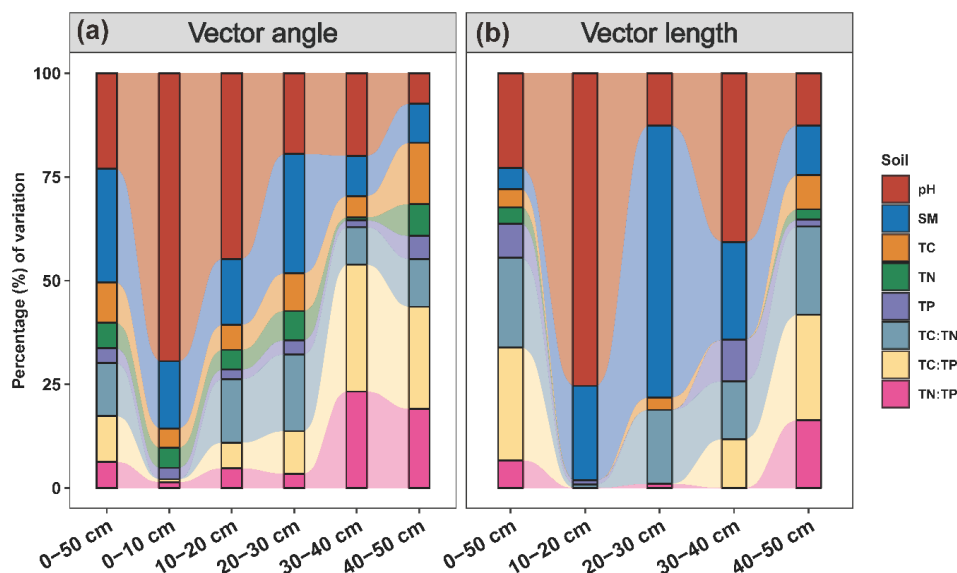
Figure 5. Influences of soil variables on vector angles (A) and lengths (B). Percentage of variation in vector angles and lengths explained by soil variables. SM, soil moisture; pH, soil acidity and alkalinity; TC, total carbon; TN, total nitrogen; TP, total phosphorus.

4. Discussion

4.1. Patterns of microbial nutrient limitation across precipitation regions throughout soil profiles

We did not observe a significant relationship between C-acquiring enzymes and precipitation (Fig. 2), and surprisingly, the vector length increased with precipitation, indicating that microbial C limitation was gradually exacerbated along the precipitation gradient in 0-50 cm. Moreover, N-acquiring enzyme activity decreased and P-acquiring enzyme activity increased with increasing precipitation (Fig. 2), which alters the stoichiometry of enzymes leading to C limitation. We attributed this pattern of microbial C limitation to

282   changes in soil nutrients and their stoichiometry across precipitation regions (Fig. 5B).

283   Microbial resource limitation is relative (Chen et al., 2018); thus, microbial C limitation can

284   depend on the combined effect of other nutrients.

285   We detected a decrease in the enzymatic C:P and N:P acquisition ratios with increasing

286   precipitation (Fig. 2). Microorganisms can shift from secreting more C-acquiring enzymes and

287   N-acquiring enzymes to secreting more P-acquiring enzymes, which implies an increased

288   demand for microbial P. Moreover, the vector angle became greater than 45° with increasing

289   precipitation (Fig. 4A). Our results notably indicated that microbial P limitation was gradually

290   exacerbated along the precipitation gradient. P is predominately derived from mineral aerosol

291   deposition and weathering, and it is highly dependent on pH (Mahowald et al., 2008; Thingstad

292   et al., 2005). High soil moisture and low soil pH increase P-acquiring enzyme activity, which

293   facilitates P solubilization (Collavino et al., 2010; Xu et al., 2020b). Our study also identified

294   that the P-acquiring enzyme was influenced by soil pH and moisture, and soil pH and moisture

295   were the main factor determining the pattern of soil enzymes along the precipitation gradient

296   (Fig. 3). Therefore, soil pH and moisture play an essential role in microbial P limitation

297   throughout soil profiles.

298   Furthermore, soil nutrients and their stoichiometry and soil physicochemical properties

299   are equally important for microbial phosphorus limitation in 0-50 cm (Fig. 5A). More C and N

300   might lead to microbial P limitation (Cui et al., 2020). This observation may be explained for

301   two reasons. On the one hand, if the C and N accumulated in the soil dilute the soil P content,

302   the microorganisms may be limited by P due to the elemental stoichiometric balance of the

303   microorganisms (Chen et al., 2017; Sinsabaugh et al., 2009; Sinsabaugh et al., 2008). Thus,

304   despite the increase in soil P, microbial P limitation remains because of the slow growth rate

305   of P relative to that of C and N. On the other hand, phosphatase molecules are rich in C and N,

306   and microorganisms require sufficient C and N to construct extracellular phosphatases

307   (Houlton et al., 2008; Treseder and Vitousek, 2001). Phosphatase may be highly responsive to

308   changes in soil elements, a critical strategy for organism adaption to human modifications of

309   the global biogeochemical cycle that can delay the onset of P limitation (Güsewell and Freeman,

310   2005; Marklein and Houlton, 2012). These findings demonstrate that precipitation regulates

311   soil physicochemical properties and nutrient status (Ma et al., 2015; Smith, 2011), which

312   consequently greatly enhances microbial C and P limitation. Our current results take a new step

313   and are valuable for understanding microbial nutrient limitation along the precipitation gradient

314   in agroecosystems.

315   *4.2. Contrasting mechanisms governing microbial nutrient limitation in topsoil and subsoil*

316   Soil properties and enzyme activities can change with soil profiles (Li et al., 2020; Luan

317   et al., 2020; Stone et al., 2014), and we observed wide differences in soil and enzyme

318   stoichiometry between the topsoil (0-20 cm) and subsoil (20-50 cm). Our study found that

319   enzymatic C:P acquisition ratios were negatively correlated with precipitation only in the

320   topsoil, which is attributable to the phosphatase distribution pattern. Enzymatic N:P acquisition

321   ratios decreased, but enzymatic C:N acquisition ratio increased along the precipitation gradient,

322   and changes substantially in the subsoil. The vector angles visualized that microbial P

323   limitation increased and responded more strongly to precipitation in the subsoil than in the

324   topsoil. In topsoil, soil pH and moisture are the major drivers of microbial P limitation (Fig.

325   5A). Soil pH governs phosphatase spatial variations and is positively correlated with enzymatic

326   C:P and N:P acquisition ratios (Xu et al., 2017). It is interesting to note that, compared to the

327   topsoil, soil nutrient availability and their stoichiometry are important factors in determining

328   microbial P limitation in subsoil (Fig. 5A). Soil substrate availability greatly influences enzyme

329   activity patterns (Stock et al., 2019), and low resources (C and N) and O_2 concentrations can

330   limit microbial metabolism (Fierer, 2017; Gu et al., 2017). Zhou et al. (2020) also found that

331   the soil C:N ratio explains a large proportion of the variation in microbial nutrient acquisition.

332   Emerging evidence suggests that subsoils, exhibit more varied organic matter sources,

333   microbial communities, and substrate availability compared to those of the topsoil (Chen et al.,

334   2019; Rumpel et al., 2012). Differences in soil properties across soil depths can lead to greater

335   environmental filtering or competition for nutrients among microorganisms than in the topsoil

336   (Bahram et al., 2015; Schlatter et al., 2020). Especially in agroecosystems, this difference

337   between topsoil and subsoil may be attributed to the agricultural tillage depth, which is

338   typically 0-20 cm. Cropland activities contribute to the homogenization of microbial

339   communities (Rodrigues et al., 2013), and alter microbial resource limitations (Cui et al., 2021).

340   On the contrary, the original soil conditions were preserved in the subsoil, resulting in microbes

341   secreting soil enzymes that are less responsive to precipitation in the topsoil than that in the

342   subsoil and attenuate the reaction of microbe nutrients limitation to precipitation in the topsoil.

343   Ecosystems cannot be limited by carbon or nutrients alone (Soong et al., 2020), and most

344   microbial communities are co-limited by energy and key nutrients (Moorhead et al., 2016).

345   Another marked trend is that the vector length increases in the subsoil with precipitation. This

346   result indicates that microbial C limitation increased in the subsoil along the precipitation

347   gradient. The detected relationships might result from that soil C:N and C:P ratios

348   progressively decline across the precipitation region in the subsoil (Fig. 1), which can lead to

349   insufficient soil C (Chen et al., 2018). In contrast, there is no relationship between microbial C

350   limitation and precipitation in topsoil. This might occur because the effect of precipitation is

351   masked by cropland activity, causing soil nutrient stoichiometry in topsoil that exhibited no

352   response to precipitation. Moreover, soil nutrients and their stoichiometry increase in their

353   interpretation of C limitation with depth (Fig. 4). Therefore, soil C:N:P stoichiometry

354   tremendously affected microbial groups involved in major biogeochemical processes (Luo et

355   al., 2020). Additionally, balanced nutrient stoichiometry is essential for maintaining microbial

356   and elemental homeostasis (Cui et al., 2019b).

357   Further, P limitation changes rapidly in the subsoil, which contributes to C limitation.

358   Nutrients are interdependent and they have a complex coupling, so changing in one nutrient

359   cycle type can alter the availability of another nutrient (Marklein and Houlton, 2012). The role

360   of P limitation in the storage capacity of the C ecosystem has previously been substantially

361   underestimated (Peng and Thomas, 2010). P affects the C-storage capacity both directly and

362   indirectly by affecting the plant growth capacity and limiting biological activities for N fixation

363   (Peñuelas et al., 2012). A model predicted less efficient microbial growth in P-limited soils,

364   indicating that P availability may affect C cycling (Waring et al., 2014). These nutrient

365   interdependencies confirm that resource limitation coupling is complex, and excess

366   precipitation leads to depletion of P and C and induces imbalances in agroecosystems. Our

367   results highlight the contrasting mechanisms governing microbial nutrient limitation in topsoil

368   and subsoil. Microbial P limitation was controlled by the soil pH and moisture in the topsoil,

369   while microbial C and P limitation in the subsoil were regulated by the soil nutrients and their

370   stoichiometry. The findings suggest that ecosystem functions and microbial nutrient limitation

371   cannot be comprehensively assessed from topsoil observations alone.

372   **5. Conclusions**

373   Understanding the key processes by which precipitation threatens microbial nutrient

374   limitation allows the assessment of nutrient trade-offs in agroecosystems, which can help us

375   meet crop production goals under change in precipitation. We detected spatial variations in soil

376   enzyme activities and stoichiometry according to the agroecosystem soil profile and along a

377   precipitation gradient. We observed an increase in microbial P limitation in the topsoil and a

378   stronger limitation of microbial C and P in the subsoil compared to the topsoil and elucidated

379   the differentiation of mechanisms among soil profiles. Our study also provides insights into the

380   change mechanism of microbial resource limitation in the context of global precipitation

381   changes and protecting vital life-support systems.

389   **Declaration of interests**

390   The authors declare that they have no known competing financial interests or personal

391   relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

392

393   **Jingjing Yang**: Conceptualization, Methodology, Software, Formal Analysis,

394   Investigation, Writing-Original Draft, **Pingting Guan**: Methodology, Formal Analysis, Data

395   Curation, Writing-Review & Editing, **Peng Zhang**: Validation, Investigation, Resources,

396   **Yunga Wu**: Investigation, Resources, Visualization, Writing-Review & Editing, **Donghui Wu**:

397   Conceptualization, Supervision, Resources, Writing-Review & Editing, Project Administration,

398   Funding Acquisition.

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