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Disconnection between plant–microbial nutrient limitation across forest biomes

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Abstract

Nitrogen (N) and phosphorus (P) availability are essential elements limiting plant-microbial growth. However, it remains unclear on whether similar or divergent patterns for plant-microbial nutrient limitations. This knowledge gap limits our capacity to predict changes in ecosystem stoichiometry and element cycling. We investigated the patterns in plant–microbial N/P limitations in forests across wide environmental gradients in China. We revealed that 42.6% of the N/P limitation between plant-microbial communities was disconnected. Patterns in plant–microbial N/P limitations were consistent only for 17.7% of N and 39.7% of P. Geospatially, the inconsistency was more evident at mid-latitudes, where plants were mainly N-limited and microbes were mainly P-limited. Furthermore, our findings are consistent with the ecological stoichiometry of plants and microbes themselves and their requirements. Particularly, the divergence in plant–microbial N/P limitations can be favored along evolutionary times to diminish their mutual competition and thus make more efficient their obligate coexistence.

1. Introduction

Nutrient availability, along with interactions with climate, vegetation, soil and topographic conditions, are known to limit plant-microbial (plant and microbial) growth, the major drivers of ecosystem function (Bar-On et al., 2018; Chen et al., 2022). However, much less is known about whether plants and microbes share similar or distinctive patterns in N and P limitation despite the well-known widespread heterogeneity in both plant and microbe nutrient limitations at the global scale (Du et al., 2020; Xu et al., 2013). This knowledge is critical for the basic understanding of ecological processes such as plant–microbe competition, C sequestration and ecosystem responses to global change and to better understand overall changes in ecosystem productivity and element stoichiometry.

As both land plant and soil microbes obtain their major nutrients from terrestrial ecosystems, convergence in N and P limitation across environmental gradients could be expected (Figure 1a) (Capek et al., 2018; Cui et al., 2022; Du et al., 2020). However, we also know that plants and microbes have distinct biogeochemical niches, ecological stoichiometric ratios (C:N:P) and efficiencies in obtaining N and P (e.g., via N atmospheric fixation and P solubilization), which may result in inconsistent N and P limitation even in the same environments (Figure 1b) (Penuelas et al., 2019). At the global scale, the leaf C:N:P molar ratio is, on average, 1212:28:1 (McGroddy et al., 2004), whereas the microbial C:N:P molar ratio is, on average, 42:6:1 (Xu et al., 2013); therefore, plants have a higher N:P demand than microbes to maintain their homeostasis. Moreover, microbes are more efficient than plants in obtaining nutrients from the soil to keep their own biological mechanisms well, as opposed to plants maintaining homeostasis in nutrient-poor ecosystems (Bardgett et al., 2003). Therefore, regardless of soil nutrient stocks and pools, plants and microbes may not have the same N and P limitations in a given location due to their different nutrient requirements and acquisition strategies. Additionally, how climate, soil depth and properties, and topographic conditions interact with plant-microbial N and P limitations may further result in important large-scale divergent patterns (Du et al., 2020; Li et al., 2014).

However, evidence for this likely divergence is lacking.

Here, we aimed to understand the N and P limitation patterns of plants and microbes across forests. To discern (1) to what extent plant-microbial N/P limitation are consistent and (2) the pathways through which climate, vegetation, soil, and topographic conditions influence plant–microbial N/P limitation consistency, we investigated plant-microbial N/P limitation and their links with climate, vegetation, soil, and topographic conditions in 183 forest ecosystems, including coniferous forests (CF), broadleaf forests (BF), and mixed forests (CBF), across different latitudinal gradients (from cold to tropical regions) and at different soil depths: organic horizon (O), eluvial horizon (A), and parent material horizon (C) in China (Figures 2a and 2b). Forests make up 31% of terrestrial ecosystems and are the largest vegetation cover on Earth, playing an irreplaceable role in CO_2 decline, faunal conservation, hydrology regulation, water eutrophication, and soil consolidation (Liu et al., 2020; Niu et al., 2016; Sardans et al., 2016; Zhang et al., 2018). In the face of increasing global warming and water pollution (Liu et al., 2022; Zhan et al., 2021), it is urgent to identify and mitigate N/P limitation and to sequester C, N, and P in forest ecosystems. Understanding whether and why the plant-microbial N/P limitation in forest ecosystems are consistent is of great importance to optimize ecological functioning and to guide the reforestation and restoration of millions of forest hectares across the globe as part of the UN Decade on Restoration (https://www.decadeonrestoration.org/).

2. Material and methods

2.1 Soil sample collection and determination

We collected soil samples from the majority of China's forest reserves, where forest ecosystems are generally less affected by human intervention (Supplementary dataset). The forest ecosystems studied included CF, BF, and CBF at 181 sampling sites, for a total sample size of 1520, and the collected soils contained the O, A, and C layers. The investigated forest ecosystems span five major climatic zones, ranging from cold-temperate to tropical. The latitude and longitude ranges of the study area are 18.9–

53.5°N and 101.0–129.7°E, respectively; the elevation range is 201–3830 m; and the annual rainfall and temperature ranges are 242–2667 mm and -5.91–23.18°C, respectively.

Soil samples were collected between July 2012 and March 2013, with each sample being a mixture of five collections from the same plot. The samples were divided into two subsamples. One was stored at 4°C to determine soil enzyme activity. The other subsample was used to determine soil physicochemical parameters, including soil moisture; temperature; bulk density; texture; pH; cation exchange capacity (CEC); base saturation (BS); C, N, and P contents; and K, Ca, Mg, Mn, Al, and Fe contents (Bao, 2005). The soil C-acquiring enzymes measured were β-1,4-glucosidase (BG) and β-D-cellobiosidase (CBH); the N-acquiring enzymes measured were β-1,4-N-acetylglucosaminidase (NAG) and L-leucine aminopeptidase (LAP); and the P-acquiring enzyme measured was acid phosphatase (AP) (Sinsabaugh and Follstad Shah, 2012).

2.2 Plant N/P limitation and climatic and topographic data acquisition

Plant N/P limitation data were obtained from the global plant N/P limitation dataset established by Du et al. (2020). The ratio of N resorption efficiency (NRE) to P resorption efficiency (PRE) was used in the dataset to refer to plant N/P limitation:

$$N(P)RE = \left(1 - \frac{mass\ of\ N(P)\ in\ senesced\ leaves}{mass\ of\ N(P)\ in\ mature\ leaves}\right) \tag{1}$$

To explain the relationship between and the consistency of plant–microbial N/P limitations, we defined plant N/P limitation as -(ln NRE/PRE) so that the positive and negative directions of plant N/P limitation and microbial N/P limitation would be consistent when the plant N/P limitation is greater than 0 for P limitation and less than 0 for N limitation (there is uncertainty in the prediction of plant N/P limits between -0.16 and 0.16).

Climatic data, including mean annual precipitation (MAP), mean annual temperature (MAT), seasonal variability of precipitation (PSEA), seasonal variability of temperature (TSEA), and potential evaporation (PET), were obtained from the WorldClim database (https://www.worldclim.org; ~1 km resolution). The aridity index (AI) was obtained using the MAP/PET ratio. Atmospheric deposition and acid rain data were obtained from the dataset established by Yu et al. (2019) for China for 1985–2015. The vegetation indices (NDVI, 2001–2015) were obtained from the Advanced Very High-Resolution Radiometer (AVHRR) developed by the Global Inventory Modeling and Mapping Study (GIMMS) team, with a resolution of 1/12° (https://ecocast.arc.nasa.gov/data/pub/gimms/3g.v1/).

2.3 Statistical analysis

The C, N, and P limitations of microbes were quantified by calculating the vector length and vector angle based on the ratio of soil enzyme activity. The vector length, representing the relative C limitation of microbes, was calculated as the square root of the sum of x^2 and y^2, where x is the relative activity of C- versus P-acquiring enzymes, and y is the relative activity of C- versus N-acquiring enzymes. The vector angle represents the microbial N/P limitation and was calculated as a tangent line extending from the plotted origin to the point (x, y) (Moorhead et al., 2015). The longer the vector length, the more severe the relative microbial C limitation, with vector angles greater than 45° representing P limitation and those less than 45° representing N limitation. Relative to a -45° angle, positive values imply P limitation, whereas negative values imply N limitation, and the positive and negative correlations are consistent with our definition of plant N/P limitation:

$$Length = \sqrt{(x^2 + y^2)} \tag{2}$$

$$Angle = atan2(x, y) \times \frac{180}{\pi} - 45 \tag{3}$$

Plant or microbial N/P limitation values greater than 0 imply P limitation, with larger values implying more severe P limitation; and values less than 0 imply N limitation, with smaller values implying more severe N limitation. When the values of both plant-microbial N/P limitations are greater than 0, it means a consistent P limitation; when the values of N/P limitation are less than 0, it means a consistent N limitation;

and when one N/P limitation value is positive and the other is negative, it means an inconsistent N/P limitation.

The variability in plant–microbial C, N, and P limitations across plant compositions and sampled soil layers was compared using the Wilcoxon test (* denotes $P < 0.05$, ** denotes $P < 0.01$, *** denotes $P < 0.001$). Linear regression was used to describe the relationship between and the consistency of plant–microbial N/P limitation; when both were in the positive and negative partitions, this implied consistent P limitation and consistent N limitation, respectively. Latitudinal patterns and breakpoints in plant–microbial N/P limitation were determined using piecewise linear regression analysis (Toms and Lesperance, 2003). The Mantel test was used to identify the effects of climate, soil, vegetation, and topography on plant–microbial C, N, and P limitations within different sampled plant compositions and soil layers (Mantel, 1967). Given that N/P limitation in microbes is less seasonally variable than that in plants, it is more valuable to estimate N/P limitation for the whole ecosystem at each site based on microbial N/P limitation and other environmental variables (Sinsabaugh and Follstad Shah, 2012). A random forest model was used to quantify the contributions of climate, soil, vegetation, topography, and microbial N/P limitation to plant–microbial N/P limitation (Breiman, 2001). Furthermore, a partial least squares pathway model was used to establish the pathway relationships between plant–microbial N/P limitation and their key drivers (all variables with loadings less than 0.7 were removed to eliminate covariates) (Russolillo, 2012). Finally, we used stepwise regression models to establish empirical formulations for coupling microbial N/P limitation with environmental variables to predict plant N/P limitation. All the above statistical analyses and plotting were performed in R software (v.4.1.3).

3 Results

3.1 Consistency of plant and microbial N/P limitation

We found microbes in most of the forest ecosystems were P limited (79.7%), and the degree of microbial N/P limitation varies by plant composition and soil layer (Figure

S1a). Although the forest ecosystem as a whole presents microbial P limitation, the P limitation was more severe in BF (6.47 ± 0.31) and CBF (7.05 ± 0.32) than in CF (3.58 ± 0.35) (Figure S1d). And the degree varies between soil layers, with less microbial P limitation in the O layer (3.97 ± 0.25) than in the A (6.66 ± 0.32) and C (7.33 ± 0.39) layers (Figure S1e). Overall, 57.8% of plants were N-limited and 42.2% of plants were P-limited in the forest ecosystems (Figure S2). Furthermore, the plant N/P limitation were inconsistent across different forest types, with plants having a significant N limitation in CF (-0.16 ± 0.01) but a not significant one in either BF (0.01 ± 0.01) or CBF (0.03 ± 0.01).

Moreover, we found that at the continental scale, the N/P limitations of plants and microbes were significantly correlated ($R^2 = 0.18$, $P < 0.001$), with 17.7% of N and 39.7% of P limitations being consistent (Figure 2c). Plant–microbial N/P limitation have a higher consistency in BF (61%; $R^2 = 0.23$, $P < 0.001$) compared to CBF (59.4%; $R^2 = 0.11$, $P < 0.001$) and CF (42.7%; $R^2 = 0.14$, $P < 0.001$) (Figure 3a). And a higher consistency, of more than 54.9%, in the O and A layers ($R^2 = 0.23$–0.25, $P < 0.001$) compared to the C layer ($R^2 = 0.15$, $P < 0.001$) (Figure 3b).

Nevertheless, 43% of Plant–microbial N/P limitation were still inconsistent, with the majority of these (40%) occurring where plants were N-limited and microbes were P-limited. Geospatially, plant and microbes maintain a consistent overall P limitation at low latitudes (18.9–32.4°N) and show a consistent P limitation alleviation with increasing latitude (Figure 4). The plant and microbial P limitations decrease with increasing latitude breakpoints at 38.0°N and 41.4°N, respectively. At latitudes greater than 32.4°N, plant P limitation shifts more rapidly to N limitation (Figure 4a), whereas microbes remain with a P limitation until 41.1°N (Figure 4b). Importantly, although plants tend towards P limitation at latitudes greater than 38.0°N, they remain with a N limitation in our observation interval (38.0–53.5°N). In contrast, microbes shift to P limitation at latitudes greater than 41.8°N.

3.2 Driving pathways for plant-microbial N/P limitation

We found that plant composition linked to climate, soil, vegetation and topographic characteristics combined to influence plant N/P limitation ($P < 0.01$). In contrast, microbial N/P limitation was less influenced by plant composition and soil layer, but rather driven by climate, soil layer, vegetation and topographic characteristics (Figure S4a). Plant N/P limitation was significantly positively correlated with microbial N/P limitations, and this consistency was regulated by climate, vegetation, soil, and topographic conditions ($R^2 = 0.999$) (Figure S4b).

Filtering variables based on correlation and machine learning and excluding covariates, we constructed a pathway model that elaborates the driving plant–microbial N/P limitation (goodness of fit = 0.563; n = 1520) (Figure 5). Plant N/P limitation was linked to microbial N/P limitation and soil characteristics, but were more importantly influenced by atmospheric deposition (total effect= 0.507) and meteorology (total effect= 0.669). Whereas microbial N and P limitation was mainly influenced by soil chemistry (total effect= -0.472). Given that microbial N/P limitation is less variable than plant seasonally, we developed a stepwise regression model that predicts plant N/P limitation by coupling environmental variables with microbial N/P limitation ($R^2 = 0.936$; n = 1520) (Table 1).

4. Discussion

4.1 Disconnected patterns in plant-microbial N/P limitations

Our study demonstrates that plant-microbial N/P limitation was inconsistent in nearly half of the cases (42.6%) and shows that when N/P limitations are inconsistent, they are often associated with plants being N-limited and microbes being P-limited (40%) (Figure 2c). These results were valid across all plant compositions and soil depths (Figures 3a and 3d). This plant–microbe inconsistency pattern is in agreement with ecosystem stoichiometry theory, suggesting that because of the different ecological stoichiometries of plants (1212:28:1) and microbes (42:6:1), microbes require a greater proportion of P than plants to maintain homeostasis (McGroddy et al., 2004; Xu et al., 2013). Thus, within the 6:1 (microbes) to 28:1 (plants) range of environmental N:P supply ratios, plant-microbial N/P limitations tend to be inconsistent, with plants being limited by N and microbes by P. We identified this key plant–microbe N/P limitation inconsistency interval to occur at 32.4–41.4°N and 41.8–53.5°N (Figure 4). Forest soil N:P ratios in this interval average 20:1 (CF) and 15:1 (BF), coinciding between plant-microbial ecological stoichiometry (Xu et al., 2013). Furthermore, we found that unlike plants that are generally N-limited as they enter mid-latitudes (38.0–41.4°N), microbes tend to shift to P-limited with increasing latitude. This is because increased cold and dry conditions inhibit microbial activity, and the decomposition of soil organic matter reduces available P acquisition (Cui et al., 2022; Zhou et al., 2020). This process exacerbates P scarcity in the ecosystem and the greater proportion of P demanded by microbes compared to plants, leading to a more pronounced shift toward P limitation in microbes while generally maintaining N limitation in plants. All these results are, thus, consistent with the higher cell growth rate of microbes than plants in general making necessary higher cell concentrations of rich-P RNA and ribosome to sustain fast amino acid assemblage and protein synthesis to sustain fast cell replication (Sterner and Elser, 2002).

We further identified that consistent plant-microbial P limitation (39.7%) prevails over consistent N limitation (17.7%) (Figure 2c) and varies across forest types and has distinct latitudinal patterns (Figures 3a and 3). With global increases in atmospheric reactive N, terrestrial ecosystems have generally received sufficient N, and excessive N deposition has also led to N saturation in forest soils (Craig et al., 2021; Liu et al., 2013). The fact that atmospheric N supplies generally increase and that P, which is normally obtained from soils, becomes scarce due to P removal from forests via erosion and harvesting, leads to an increasing separation in N and P supplies, ultimately making P limitation more prevalent in forest ecosystems. Geospatially, we found that consistent P limitation was more prevalent in BF (44.5%) and CBF (46.2%) than in CF (14.8%) (Figure 3a). This because BF and CBF were mostly located at low and middle latitudes (18.9–32.4°N) and had higher primary productivity, requiring more nutrients to grow, whereas their soils were relatively poor, which exacerbated P limitation (Du et al., 2020;

Xu et al., 2013). In contrast, consistent N limitation was more prevalent in CF (27.9%), which was typically distributed at high latitudes where plant-microbial growth was slow and soil nutrients were abundant, mitigating P limitation. Moreover, the average soil N:P ratio was 16:1, and the plant N:P ratio was 22:1 in CF, whereas the average soil N:P ratio was 33:1 and the plant N:P ratio was 28:1 in BF (McGroddy et al., 2004; Xu et al., 2013). These values mean that plants in CF receive relatively little N from the soil, which further leads to competition for soil N between plants and microbes, thus making the whole ecosystem N-limited.

The disconnection in plant-microbial N/P limitation was also influenced by the soil layer, with a greater disconnection in layer C (R^2=0.15, $P < 0.001$) than in layers O-A (R^2=0.23-0.25, $P < 0.001$) (Figure 3b). Here, nutrients are partly used by the microbes and partly taken up by plant roots, and the two interact closely in the O-A layers (Capek et al., 2018), so microbial and plant N/P limitation tend to be consistent. In contrast, plants generally obtain their nutrients from the O-A layers rather than from the C layer, so the microbial N/P limitation in the C layer is relatively inconsistent with the plant N/P limitation.

4.2 Divergent drivers of plant-microbial N/P limitation

Meteorological conditions coupled with atmospheric N deposition are key in determining plant N/P limitation. In contrast, microbial N/P limitation was better explained by soil chemistry (Figure 5). Atmospheric deposition was the most important pathway of influence ($R = 0.47$, $P < 0.001$), and there is an increasing number of reports that atmospheric reactive N converts N limitation to P limitation in forest ecosystems (Asner et al., 2015). Our study further shows that the intensity of plant-microbial responses to the intensified atmospheric deposition of N was not uniform, with plant N/P limitation being more responsive than microbial N/P limitation (total effect; 0.507 vs. 0.214) (Figures 5b and S6). This means that as atmospheric deposition of nutrients intensifies, it can lead to a disconnection in plant–microbial N/P limitation. In contrast, microbial N/P limitation is more influenced by soil chemistry than plant N/P limitation (total effect; -0.472 vs. -0.175). As soil acidification/salinization and soil nutrient deficiencies increase, the microbial response is more rapid, while plants are largely able to obtain nutrients from atmospheric deposition, leading to a disconnection in plant–microbial N/P limitation.

Furthermore, given the potential disconnection in plant–microbial N/P limitation and the fact that microbial N/P limitation is less seasonally variable than plant N/P limitation (Sinsabaugh and Follstad Shah, 2012), we developed equations to predict plant N/P limitation based on microbial N/P limitation and environmental variables to help better evaluate the extent of whole-ecosystem N/P limitation (Table 1).

4.3 Ecological impacts and implications

Our work suggests that predicting ecosystem stoichiometry requires the investigation of multiple organisms simultaneously, as these organisms not only support different stoichiometries but also disconnect N and P limitation patterns. Whereas in the past, plant-microbial N/P limitation and their relationship to the C, N, and P cycles have generally been studied independently. Our work demonstrates variation in the degree and direction of N/P limitation between plants and microbes. This finding indicates the uncertainty in C, N and P sequestration predicted by individual N/P limits and that soil nutrient amendments based on individual N/P limits may also lead to divergent nutrient mitigation. For example, our study shows a 42.6% inconsistency in plant–microbe N/P limitation, mostly due to microbes being P-limited and plants being N-limited, in which case P addition targeting microbial P limitation may further enhance plant N limitation, and similar mechanisms are present when considering only individual plant nutrient mitigation.

Furthermore, global atmospheric N deposition trends continue to change dynamically, increasing in regions including East Asia and southern Brazil and decreasing in Europe (Ackerman et al., 2019). The net soil P loss rate is approximately 5.9 kg ha^{-1} yr^{-1} and, in particular, upward of 8.9 kg ha^{-1} yr^{-1} in China and South America (Alewell et al., 2020). An increase in N deposition and an irreversible (very slow P cycling) decrease in soil P led to more prevalent trends in P limitation in forest ecosystems globally. Specifically, in ecosystems where plants and microbes are consistently N-limited, plants will overcome N limitation faster than microbes will because plants respond more strongly to N deposition. This will result in the decoupling of the plant–microbial N/P limitation in the short term and eventually in both plants and microbes converging toward P limitation. In contrast, ecosystems in which plants and microbes are consistently P-limited and those in which plants are N-limited and microbes are P-limited (which accounts for the vast majority of the inconsistency) will consistently have plant–microbe P limitation. The dynamics of N and P supply to terrestrial ecosystems will therefore lead to corresponding changes in the consistency of plant–microbial N/P limitation. The ecological functions resulting from these changes need to be quantified, for example, in terms of spatial response to C sequestration and nutrient storage in plants and microbes.

Our findings also indicate a clear connection of the results with what would theoretically be expected in the frame of ecological stoichiometry, in particular, with the growth rate hypothesis, and further suggest that divergence in N and P requirements between coexisting plant and microbe communities can be favored along evolutionary times to diminish their mutual competition pressure to make more efficient and possible their obligate coexistence given their general complementary and mutual dependent functions. All this is consistent with the general trend of divergence in biogeochemical niche among different clades along evolutionary time (Peñuelas et al., 2019).

In summary, we highlight the continental-scale disconnection in plant-microbial N/P limitation and the associated uncertainties in assessing ecological functions based on individual plant or microbial N/P limitations. Targeted mitigation measures that take into account the divergent patterns of plant-microbial N/P limitation, and future environmental trends can advance the understanding of ecosystem function. It is essential to enhance terrestrial primary productivity by explicitly considering the continental-scale disconnected plant-microbial N/P limitation under changing climate scenarios, with the expectation of sequestering more C, N and P in terrestrial soils.

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Competing interests: The authors declare that they have no competing interests. Data and materials availability: All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Requests for additional materials and database should be addressed to Ji Liu (liuji17@mails.ucas.ac.cn) and Linchuan Fang (flinc629@hotmail.com).

Figure captions

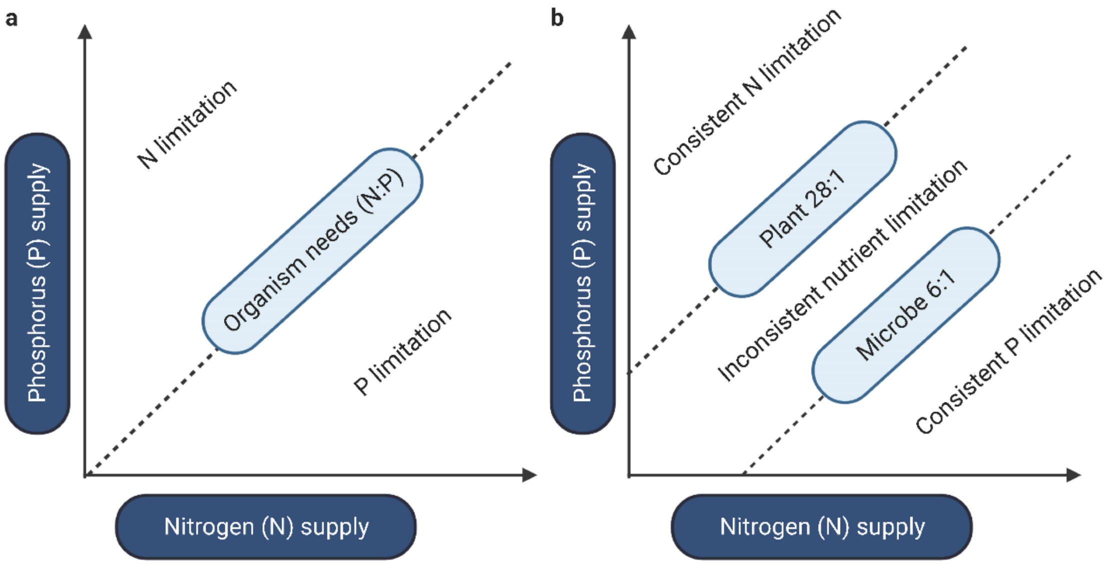
Figure 1. The concept of plant-microbe nutrient limitation. Organisms are usually limited by nitrogen (N) or phosphorus (P), because of differences in N and P supply to needs ratios(a); Plant and microbe have potentially divergent nutrient limitations due to their own ecological stoichiometry and nutrient acquisition strategies.

Figure 2. Sample site spatial distribution (a), biome distribution (b), and plant–microbial nitrogen (N)/phosphorus (P) limitation (c).

Figure 3. Plant-microbial nitrogen (N)/phosphorus (P) limitation in different plant compositions (c) and soil layers (d) (n = 1520). Plant compositions: CF, coniferous forest; CBF, coniferous-broadleaf mixed forest; BF, broadleaf forest. Soil layers: O, organic horizon; A, eluvial horizon; C, parent material horizon.

Figure 4. Latitudinal patterns and breakpoints of plant–microbial nitrogen (N)/phosphorus (P) limitation. The breakpoint of plant N/P limitation is 38°N (97.5% confidence interval: 37.9–38.6°N) (a), and the breakpoint of microbial N/P limitation is 41.4°N (97.5% confidence interval: 40.8–42.3°N) (b).

Figure 5. The partial least squares pathway model (PLS-PM) disentangles the main pathways of influence of key climate, vegetation, topography, and soil attributes on plant-microbial nitrogen (N)/phosphorus (P) limitations (a) and the effects of these variables (b) (n = 1520); R^2 indicates the variance in the dependent variable explained by the model; *** represents significant effects at $P < 0.001$. MAP, mean annual precipitation; MAT, mean annual temperature; PSEA, seasonal variability of precipitation; TSEA, seasonal variability of temperature; PET, potential evaporation; AI, aridity index; BS, base saturation.



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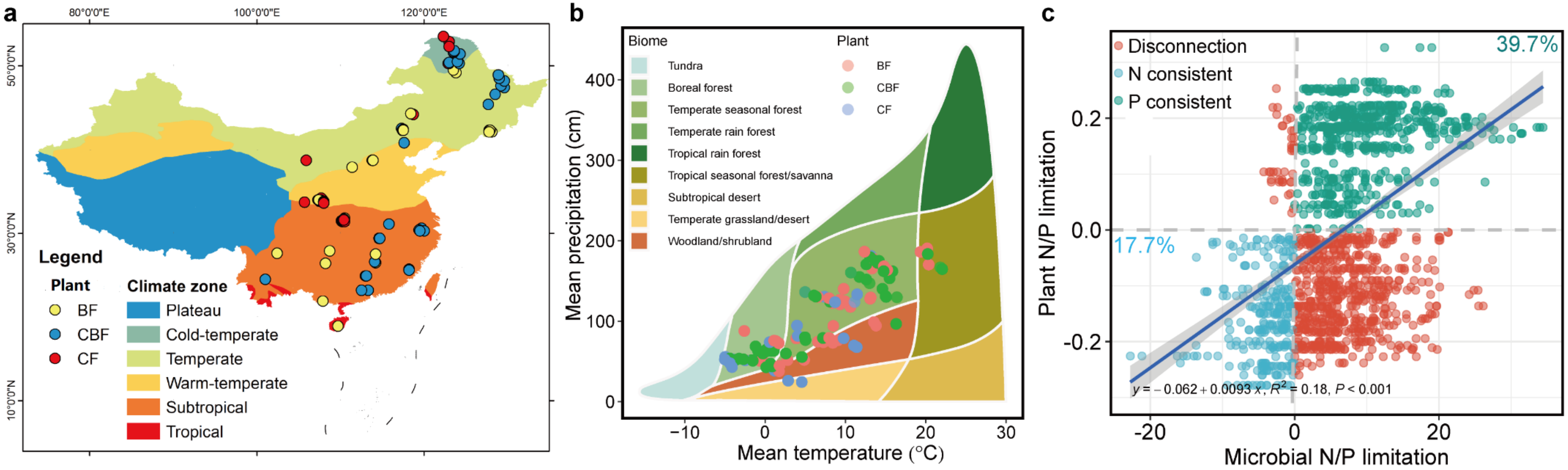

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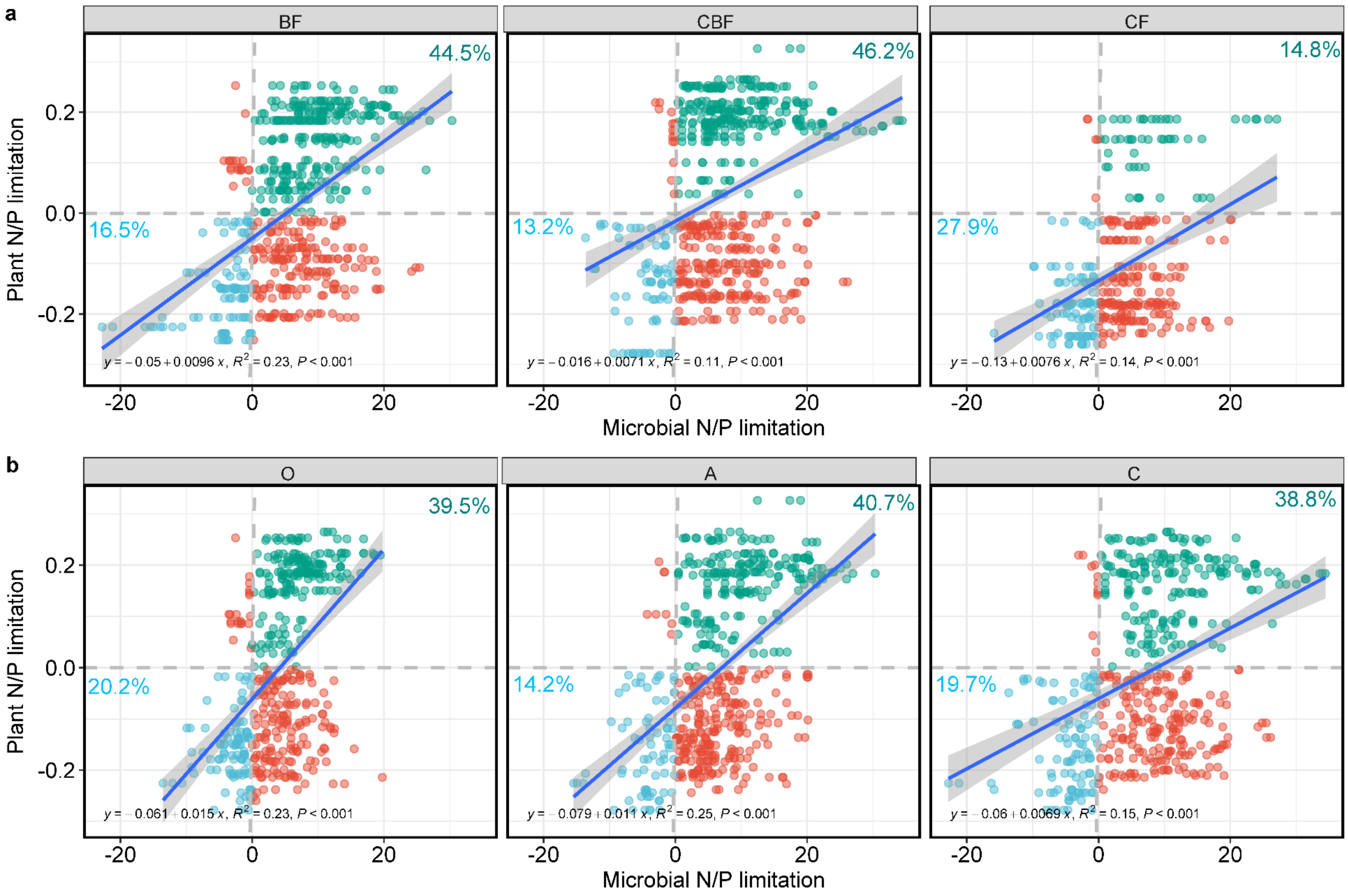
a
BF
CBF
CF
44.5%
16.5%
46.2%
13.2%
14.8%
27.9%
Plant N/P limitation
0.2
0.0
-0.2
y = −0.05 + 0.0096 x, R² = 0.23, P < 0.001
y = −0.016 + 0.0071 x, R² = 0.11, P < 0.001
y = −0.13 + 0.0076 x, R² = 0.14, P < 0.001
-20
0
20
Microbial N/P limitation
b
O
A
C
39.5%
20.2%
40.7%
14.2%
38.8%
19.7%
Plant N/P limitation
0.2
0.0
-0.2
y = −0.061 + 0.015 x, R² = 0.23, P < 0.001
y = −0.079 + 0.011 x, R² = 0.25, P < 0.001
y = −0.06 + 0.0069 x, R² = 0.15, P < 0.001
-20
0
20
Microbial N/P limitation

Figure 3. Plant–microbial nitrogen (N)/phosphorus (P) limitation in different plant compositions (c) and soil layers (d) (n = 1520). Plant compositions: CF, coniferous forest; CBF, coniferous-broadleaf mixed forest; BF, broadleaf forest. Soil layers: O, organic horizon; A, eluvial horizon; C, parent material horizon.

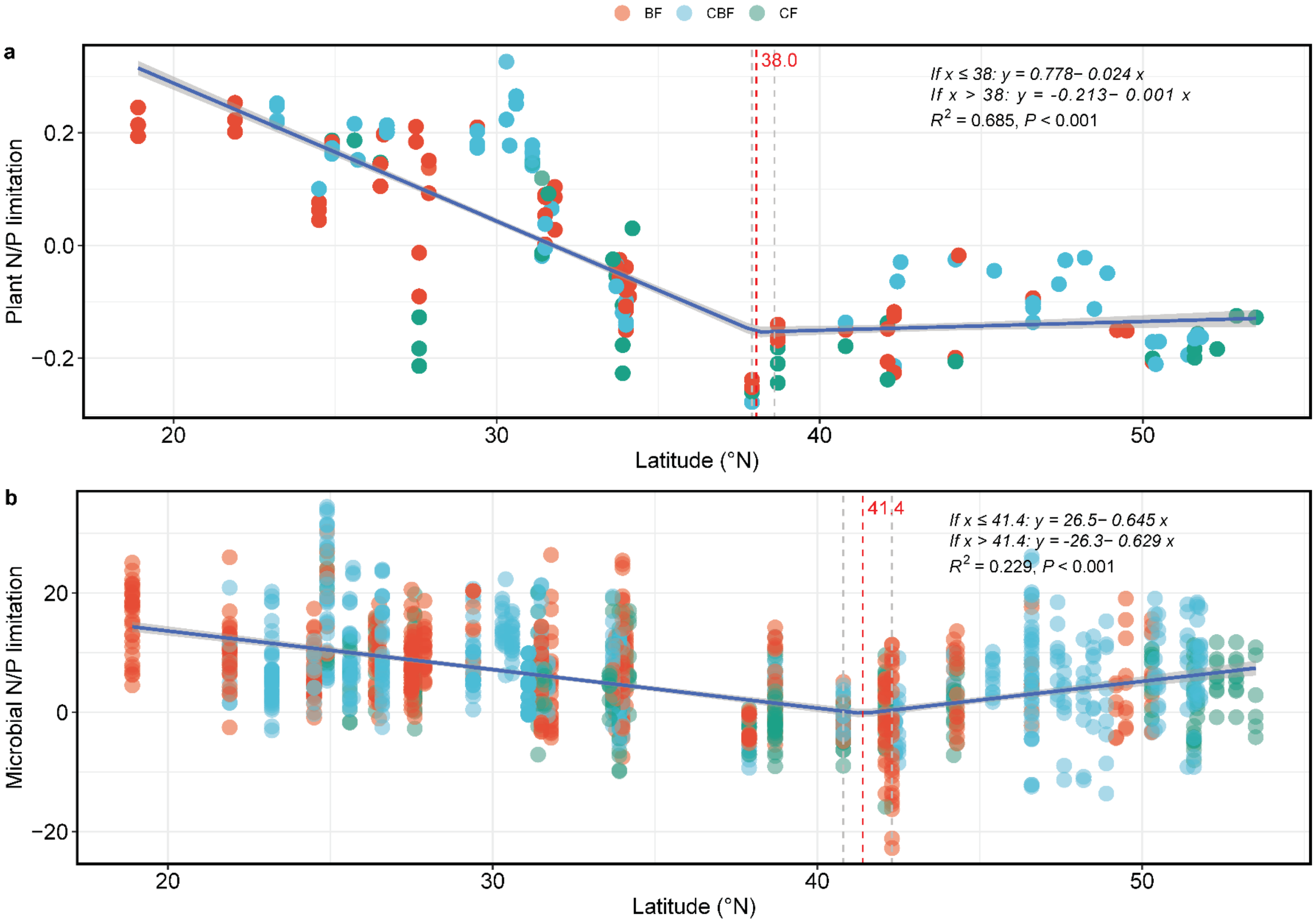● BF ● CBF ● CF
a
Plant N/P limitation
0.2
0.0
−0.2
38.0
If x ≤ 38: y = 0.778− 0.024 x
If x > 38: y = -0.213− 0.001 x
R^2 = 0.685, P < 0.001
20
30
40
50
Latitude (°N)
b
Microbial N/P limitation
20
0
−20
41.4
If x ≤ 41.4: y = 26.5− 0.645 x
If x > 41.4: y = -26.3− 0.629 x
R^2 = 0.229, P < 0.001
20
30
40
50
Latitude (°N)

Figure 4. Latitudinal patterns and breakpoints of plant–microbial nitrogen (N)/phosphorus (P) limitation. The breakpoint of plant N/P limitation is 38°N (97.5% confidence interval: 37.9–38.6°N) (a), and the breakpoint of microbial N/P limitation is 41.4°N (97.5% confidence interval: 40.8–42.3°N) (b).

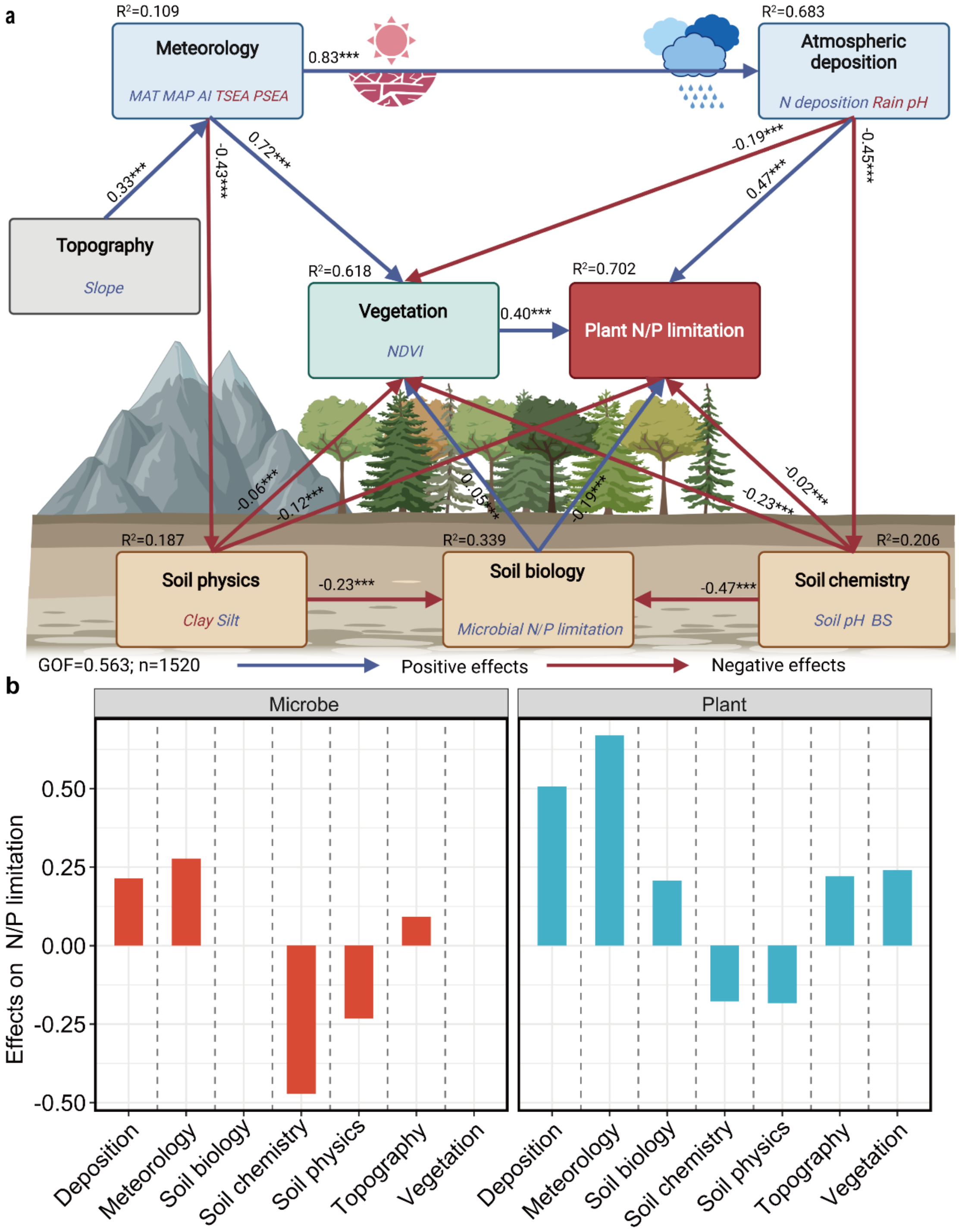

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Table 1. Stepwise multiple regression fitting for plant nitrogen/phosphorus limitation ($R^2 = 0.936$; n = 1520).

	Estimate	Std. Error	Pr (>|t|)
Intercept	-0.465	0.041	***
Slope	-0.001	0.000	***
MAT	0.017	0.001	***
MAP	1.85E-04	1.40E-05	***
AI	-7.27E-06	1.16E-06	***
TSEA	2.36E-04	8.50E-06	***
PSEA	-0.002	0.000	***
Rain pH	0.017	0.005	***
Silt	0.001	0.000	***
Clay	0.001	0.000	***
BS	2.96E-04	5.48E-05	***
Microbial N/P limitation	0.001	0.000	***
Soil pH	-0.004	0.001	**
NDVI	0.108	0.017	***

Note: * represents significant effects at $P < 0.05$; ** represents significant effects at $P < 0.01$; *** represents significant effects at $P < 0.001$. MAP, mean annual precipitation; MAT, mean annual temperature; PSEA, seasonal variability of precipitation; TSEA, seasonal variability of temperature; PET, potential evaporation; AI, aridity index; BS, base saturation; NDVI, normalized difference vegetation index.

Supplementary figure captions

Figure S1. Soil enzyme activity characteristics (a) and microbial carbon (C) and nitrogen (N)/phosphorus (P) limitations in different forest types (b, d) and soil layers (c, e) (n = 1520). C-acquiring enzymes: β-1,4-glucosidase (BG) and cellobiohydrolase (CBH); N-acquiring enzymes: β-1,4-N-acetylglucosaminidase (NAG) and L-leucine aminopeptidase (LAP); P-acquiring enzyme: alkaline or acid phosphatase (AP). Plant compositions: CF, coniferous forest; CBF, coniferous-broadleaf mixed forest; BF, broadleaf forest. Soil layers: O, organic horizon; A, eluvial horizon; C, parent material horizon; * denotes $P < 0.05$, ** denotes $P < 0.01$, *** denotes $P < 0.001$.

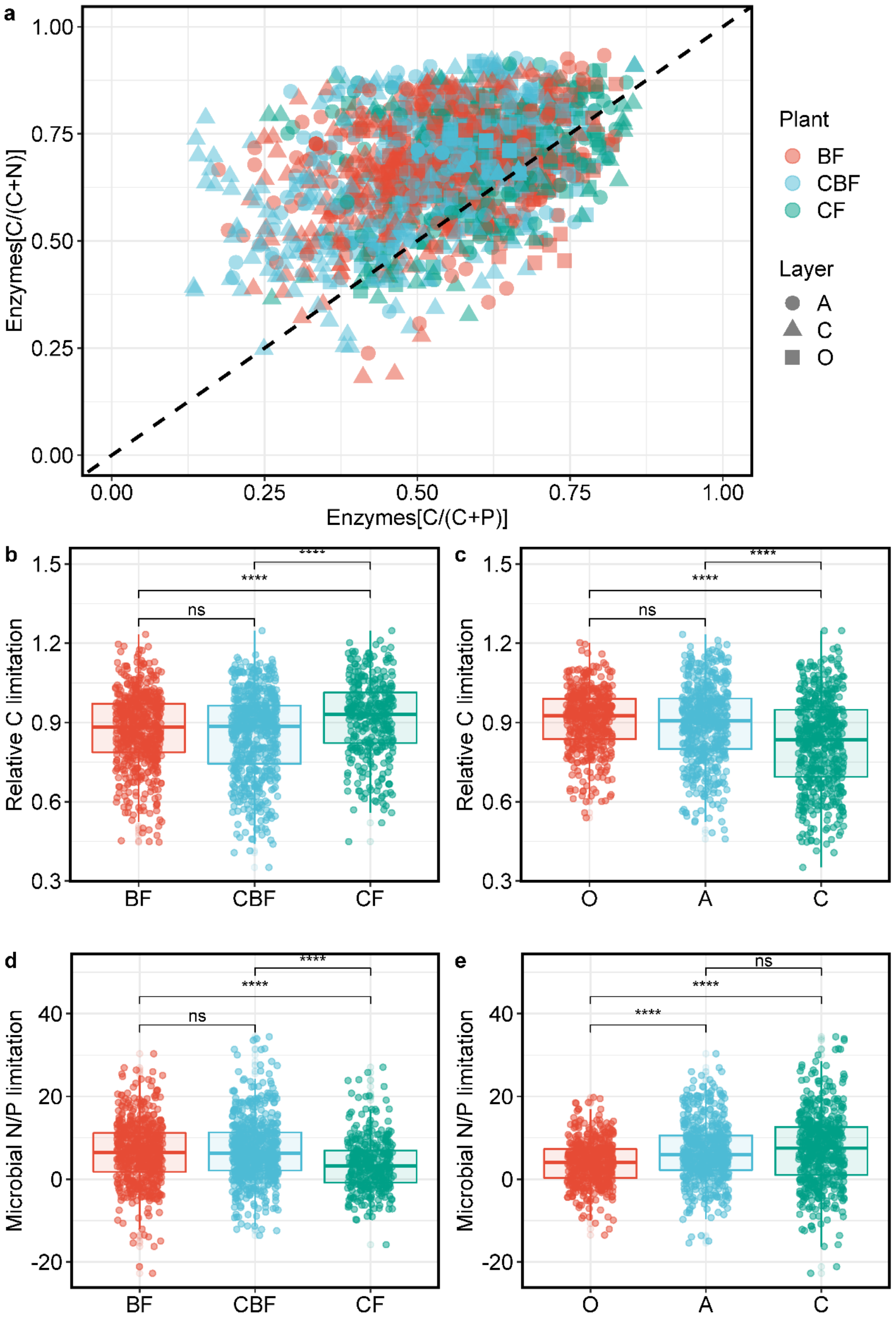
Figure S2. Plant nitrogen (N)/phosphorus (P) limitation characteristics in different plant compositions (n = 1520). Plant compositions: CF, coniferous forest; CBF, coniferous-broadleaf mixed forest; BF, broadleaf forest. * denotes $P < 0.05$, ** denotes $P < 0.01$, *** denotes $P < 0.001$.

Figure S3. Plant–microbial nitrogen (N)/phosphorus (P) limitation linkages in different climatic zones.

Figure S4. Pairwise comparisons of the relationships between plant species and soil layers sampled to determine the association between plant–microbial carbon (C) and nitrogen (N)/phosphorus (P) limitations and environmental variables (n = 1520) (a). Color gradients indicate Spearman’s significance, and line gradients represent Spearman’s correlation coefficients. Identification of the relative contribution of environmental variables to plant N/P limitation by a random forest model (n = 1520) (b); * denotes $P < 0.05$, ** denotes $P < 0.01$, *** denotes $P < 0.001$. MAP, mean annual precipitation; MAT, mean annual temperature; PSEA, seasonal variability of precipitation; TSEA, seasonal variability of temperature; PET, potential evaporation; AI, aridity index; Tsoil, soil temperature; CEC, cation exchange capacity; BS, base saturation; Soil C:N, soil carbon to nitrogen ratio; Soil C:P, soil carbon to phosphorus ratio; Soil N:P, soil nitrogen to phosphorus ratio.

Figure S5. Loading of the variables in the partial least squares pathway model.

Figure S6. Direct and indirect effects between modules in the partial least squares pathway model.



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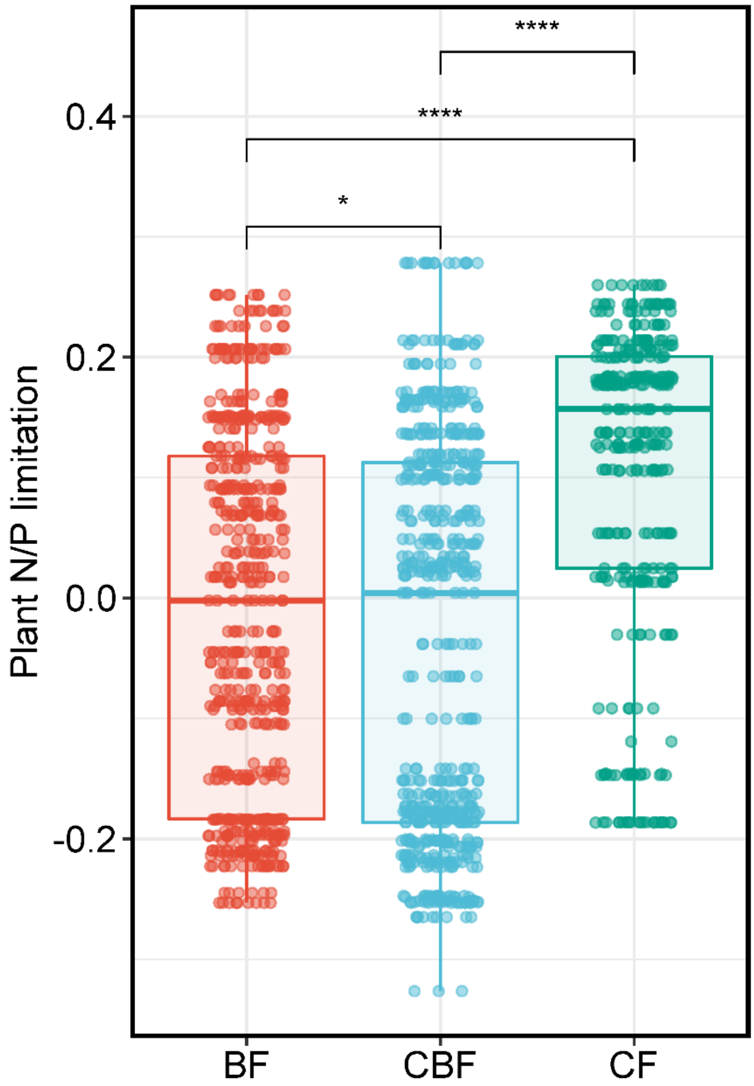

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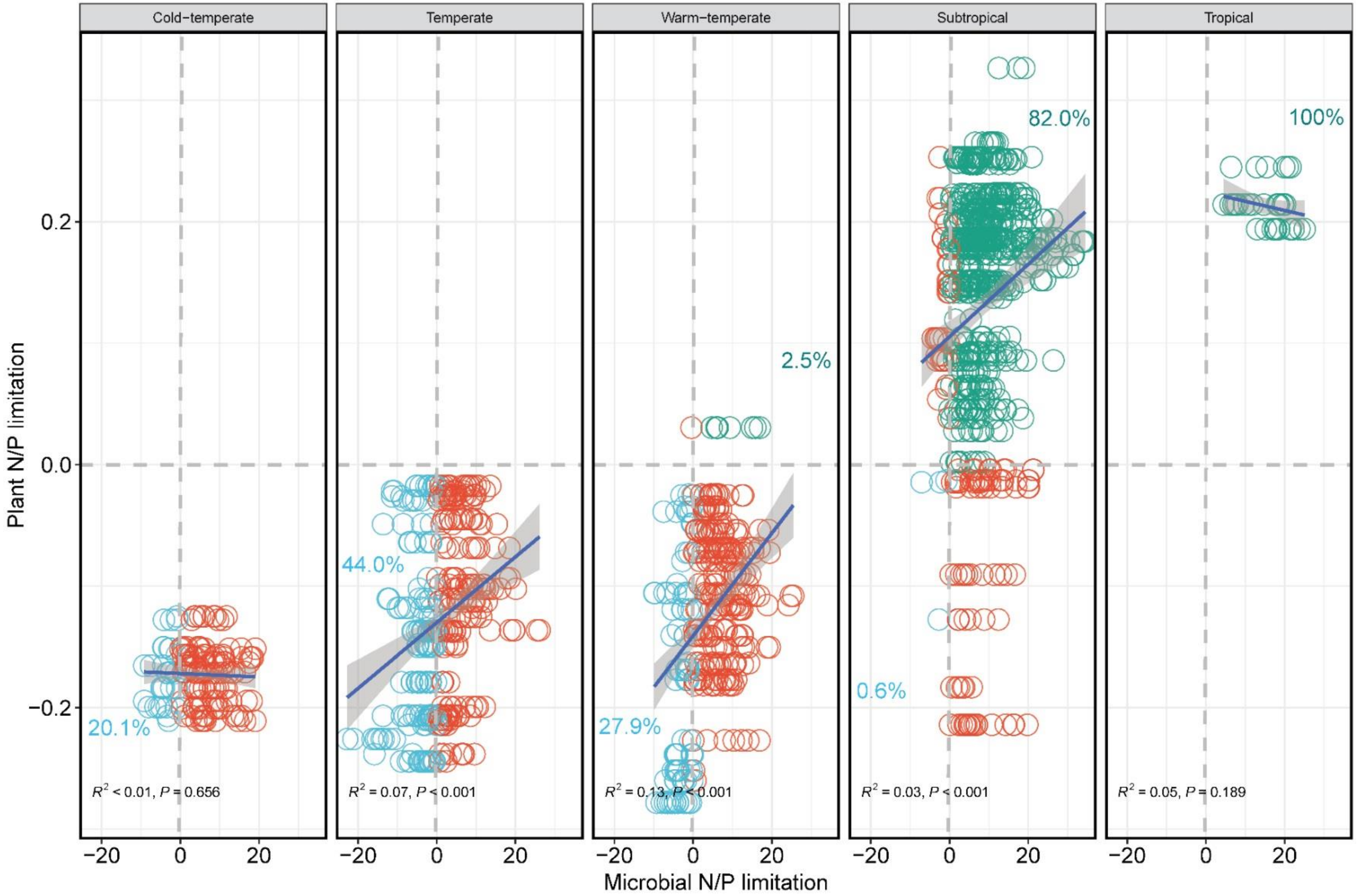

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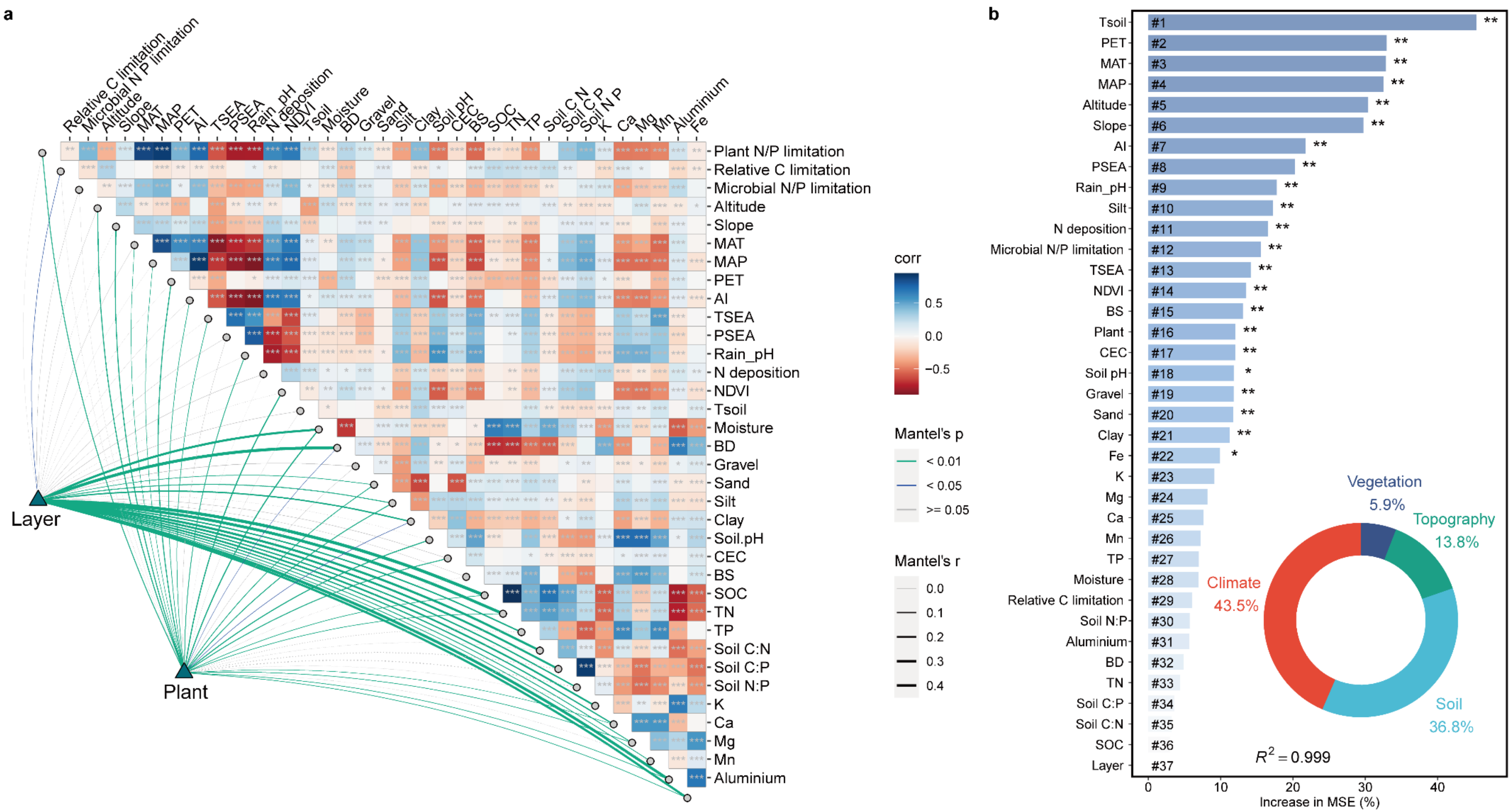

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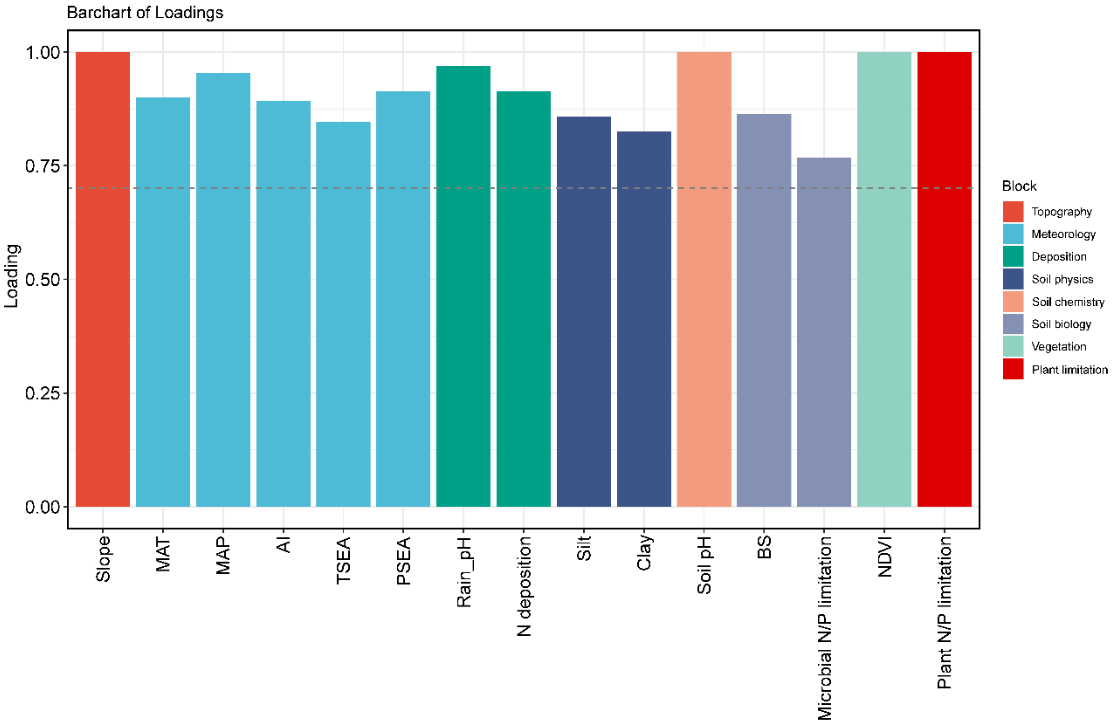

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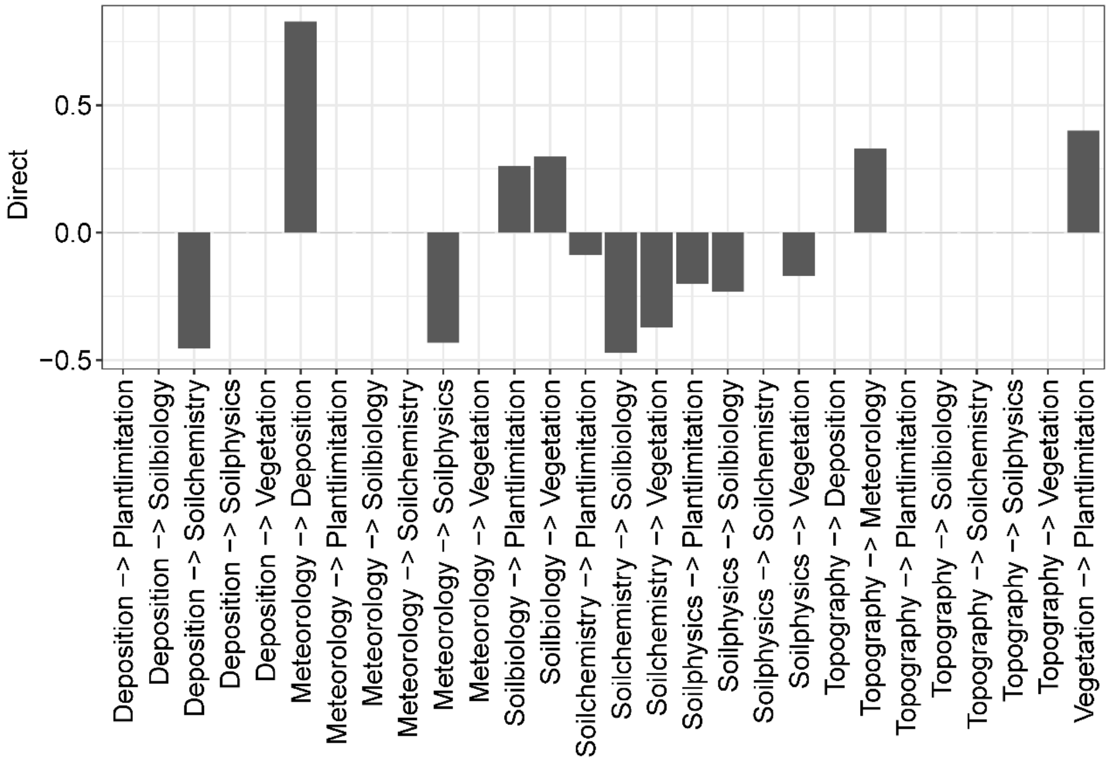
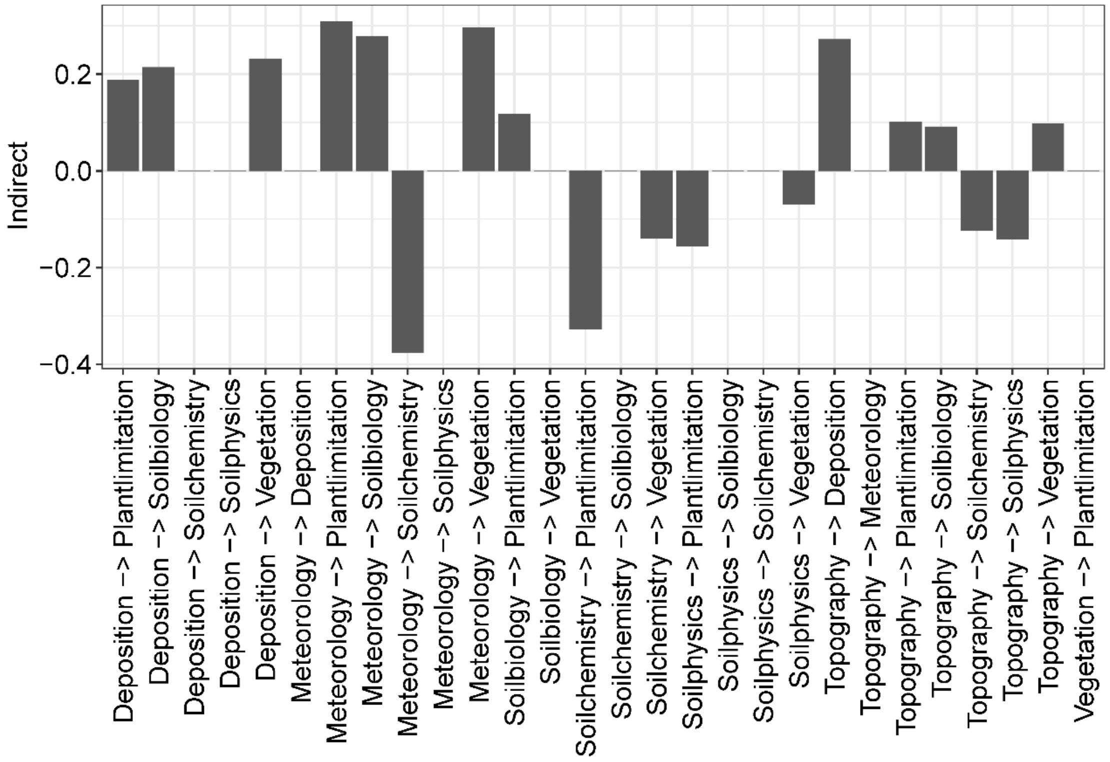
a Barchart of direct effects



b Barchart of indirect effects



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