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Shifts in chemical and microbiological properties belowground of invader *Ageratina adenophora* along an altitudinal gradient

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Abstract

Tropical mountain ecosystems are usually colonized by numerous invasive plant species and represent an ideal 'natural laboratory' to study the effects of altitude on plant invasion. The aim of this study was to investigate the soil chemical and microbiological properties along an altitudinal gradient on a mountain colonized by the invader *Ageratina adenophora*. Rhizosphere soil of A. adenophora was collected over an altitudinal gradient (1400–2400 m) in Ailao Shan, China. We determined soil organic carbon (C), nutrient contents, enzyme activities, bacterial community composition as well as C and nitrogen (N) contents of the plant roots. Ecoenzymatic stoichiometric indices were calculated to estimate the relative C, N or P limitations of the microbial community. There was a significant effect of altitude on soil organic C in the rhizosphere, and a turning point in these measured variables was detected at an altitude of 2000 m. At low elevations, the rapid growth of invasive plants depleted the deficient phosphorus (P) in tropical soils, leading to microbial P limitation; at high elevations, microbes invested more energy to obtain C from resistant litter, leading to microbial C limitation. Bacterial beta diversity and soil pH contributed most to the altitudinal differences in ecoenzymatic stoichiometry, and Proteobacteria and Acidobacteria were the dominant bacterial phyla that determined the nutrient uptake status of microorganisms. These results demonstrate how microbial nutrient acquisition belowground of *A. adenophora* along an altitudinal gradient, which could contribute to further knowledge about the effects of altitude on biological invasion.

Keywords *Ageratina adenophora*, nutrients, ecoenzymatic stoichiometry, microbial metabolic limitation, bacterial community

入侵植物紫茎泽兰根围土壤化学及微生物属性海拔变化格局

摘要：热带地区山地生态系统是外来植物入侵的重要区域，是研究外来植物扩散机制的“天然实验室”。本研究试图探明入侵植物紫茎泽兰(*Ageratina adenophora*)根围土壤化学(pH及土壤养分)和微生物(酶活性和细菌群落)特性沿海拔梯度的变化规律。本研究以哀牢山(1400–2400 m)不同海拔梯度分布的紫茎

泽兰为研究对象，采集根围土，测定土壤有机碳及养分含量，以及植物根系碳和氮含量。分析与土壤有机碳、氮及磷循环的酶活性，通过计算土壤酶化学计量参数，探究微生物生长代谢利用碳、氮及磷的规律。借助高通量测序技术对16S rDNA的V4区测序，分析细菌群落结构。研究结果显示，海拔显著影响紫茎泽兰根系氮及及其根围土壤有机碳含量，且这些测量指标在海拔2000 m出现拐点。处在低海拔，入侵植物快速生长耗竭土壤中相对缺乏的磷，磷素是限制微生物生长的重要养分元素；而在高海拔，微生物需要投入更多的能量降解有机质获取碳，导致微生物生长的碳限制。细菌群落β多样性及pH是决定不同海拔酶化学计量参数差异的重要因子；变形菌门和酸杆菌门是决定微生物养分利用状况的主要细菌门类。这些结果阐明了不同海拔梯度上紫茎泽兰根围土壤微生物的养分利用规律，有助于认识入侵植物沿海拔扩散机制。

关键词：紫茎泽兰(*Ageratina adenophora*)，养分，生态酶化学计量数，微生物代谢限制，细菌群落

INTRODUCTION

The global temperature is increasing, which increases the risk of exotic plants spreading to higher elevations (Gu *et al.* 2021). At the regional scale, elevation redistributes the two most important climatic factors, temperature and precipitation (Jobbagy and Jackson 2000). Not only climatic factors such as temperature and precipitation but also soil nutrients determine the distribution of plants along an elevational gradient, which are key factors for colonization (Concilio *et al.* 2017). At lower elevations, where temperature and precipitation are relatively high, both are beneficial to plant growth, resulting in high nutrient consumption. However, at higher elevations, and thus lower temperatures, plant more slowly and consumes fewer nutrients. Soil nutrient status and cycling are closely linked to the composition and activity of the soil microbial community (Shigyo *et al.* 2019; Xu *et al.* 2015). Studying the variations in soil nutrients, microbial communities and their activities during the upward spread of plants along altitudinal gradients can provide a theoretical basis for assessing the risk of spread of invasive plants.

Microorganisms acquire nutrients by secreting specific extracellular enzymes to degrade organic matter in the soil (Sinsabaugh *et al.* 2009). The activity of enzymes involved in the degradation of specific nutrients suggests that the allocation of microbial energy to obtain the relevant nutrients corresponds to the lack of such mineral elements for microbial metabolism (Sinsabaugh *et al.* 1994). Microbial resource acquisition could be assessed by calculating the stoichiometry of enzymatic activities (Sinsabaugh *et al.* 2008, 2009). Moorhead *et al.* (2013) proposed a method calculating vectors of ecoenzymatic activities to assess the nutritional status of the microbial community. This method converted the relative investment in C versus nutrient acquisition or P versus N acquisition into relative community resource requirements and provided clear metrics of relative C limitation and relative P versus N limitation (Moorhead *et al.* 2016). Cui *et al.* (2021) found that the C and P limitations were higher at high altitudes (3000–3500 m) than at low altitudes (2800 m), which might be due to the changes in soil temperature and moisture along the altitudinal gradient. Further analysis of the stoichiometric enzymatic activity ratios in the soil around an invader along an altitude gradient could provide a clue to the status of microbial nutrient acquisition.

Soil bacteria are ubiquitous and exhibit enormous numbers and wide functional diversity, which is one of the most important factors in nutrient cycling. In general, higher elevations correspond to lower soil temperatures but higher soil organic matter and nutrient contents compared with lower elevations (Siles *et al.* 2017), which is an important factor in bacterial community composition and microbial nutrient acquisition. Some studies have suggested that soil microbial communities in alpine ecosystems suffer from relative C and phosphorus (P) limitations despite high soil nutrient levels (Cui *et al.* 2021). It can be concluded that there is a clear correlation between bacterial community composition and microbial nutrient use, but it is not clear how this correlation changes with increasing elevation and whether it is related to soil factors.

Ageratina adenophora (Spreng.) King & H.Rob. is an herbaceous, perennial, triploid Asteraceae native to Mexico; it was first accidentally introduced to Yunnan, China, around 1940 (Qiang 1998). Currently, it is one of the most notorious exotic plants in Southwest China because of its strong colonizing and spreading ability, threatening native biodiversity (Wan *et al.* 2010).

The objective of the present study was to analyse variations in microbial nutrient uptake belowground of invader *A. adenophora* along an altitudinal gradient on the Ailao Shan in Yunnan Province. On the Ailao Shan, there was a significant change in vegetation type from monsoon evergreen bread-leaved forest to upper montane evergreen bread-leaved forest at 2100 m (Zhu *et al.* 2019). We hypothesized that (i) With increasing altitude, there is a clear transition in soil nutrients and bacterial community composition on a certain altitudinal gradient corresponding to the shift in microbial nutrient limitation; (ii) Soil chemical metrics and bacterial community composition along an altitudinal gradient contributed to different nutrient limitations of microorganisms.

MATERIALS AND METHODS

Sampling sites

In this study, the rhizosphere soil of *A. adenophora* was collected along an altitudinal belt from 1400 to 2400 m on Ailao Shan. This mountain (N 24.00′–24.44′, E 100.54′–101.29′) is located in Jingdong County, Yunnan Province, China. The average annual rainfall is 1931 mm, the average annual evaporation is 1485 mm and the average annual temperature is 11.3 °C (Qi *et al.* 2021). Sampling sites were selected at an elevation interval of 200 m, along with three replicates for each elevation gradient. The distance between these replicates was ~10 m, and a total of 18 soil samples were collected. From the distribution of *A. adenophora* on the sunny slope, three to five plants (approximately 1 m intervals between each plant) were randomly selected and removed to collect the soil shaken from the root; these were combined into one sample. The soil samples and plant roots were brought back to the laboratory, and the fine roots and fallen objects in the soil were removed and passed through a 2-mm sieve. The root samples were cleaned and dried and then crushed through a 0.15-mm sieve for carbon and nitrogen determination. Part of the soil sample was air-dried for soil nutrient and pH determination; another part was stored in a 4 °C refrigerator for soil enzyme activity determination; and the final part was stored at −40 °C for soil DNA extraction.

Soil nutrients and pH assay

Soil pH was measured at a water-to-soil ratio of 2.5:1; soil organic C content was determined by the volumetric $K_2Cr_2O_7$–H_2SO_4 oxidation method under high-temperature heating; total nitrogen was determined by the semi-micro Kjeldahl method; and total phosphorus was measured by HF–$HClO_4$ digestion (Lu 1999). The organic C and N contents of the plant roots were measured by an elemental analyser (Vario MAX CN, Germany).

Determination of soil enzyme activities

Enzyme activity was measured by the microplate fluorescence method, and 4-methylumbelliferone (MUB) was used as a labelled substrate to measure the activity of soil β-glucosidase, β-*N*-acetylglucosaminidase, aminopeptidase and acid (alkaline) phosphatase. These four enzymes are identified as the typical representative indicators of C, N, and P acquisition for microorganisms and are commonly used in calculations of ecoenzyme stoichiometry (Sinsabaugh *et al.* 2008). The measures of enzyme activities have been described at length by DeForest (2009). First, 1 g of fresh soil was weighed and mixed in 125 mL of 50 mmol L^{-1} acetate buffer to prepare a uniform suspension. Then, a black 96-well plate was used for each sample with eight replicates; each plate contained 200 μL of buffer in each of the three columns of wells on the plate, and 50 μL of buffer, 10 μmol L^{-1} MUB and 200 μmol L^{-1} substrate was added to each well sequentially, which are the blank control, standard reference and substrate control. Following these columns, 200 μmol L^{-1} of soil slurry was added to each well in another three columns, and then 50 μL of 200 μmol L^{-1} substrate, buffer and 10 μmol L^{-1} MUB were pipetted in turn, which was set as the soil sample, sample control and standard control, respectively. After that, the samples were immediately incubated at 20 °C in the dark for 2 h. Finally, a multifunctional microplate reader was used to measure the fluorescence value under 365 nm excitation light and 450 nm transmitted light.

Illumina sequencing analysis of 16S rRNA gene amplicons

Total genomic DNA was extracted from 0.5 g of fresh soil samples using a FastDNA™SPIN kit (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's protocol. DNA concentration and purity were monitored on 1% agarose gels. Depending on the concentration, DNA was diluted to 1 ng μL^{-1} with sterile water. The V4 region of the 16S rRNA gene was PCR amplified using primers 515F and 806R, which contained a barcode, and sequencing was performed on an Illumina HiSeq platform to generate 250 bp paired-end reads.

Paired-end reads from the original DNA fragments were fused using FLASH (Magoč and Salzberg 2011) and then assigned to each sample based on the unique barcodes. Sequences were analysed using the Quantitative Insights Into Microbial Ecology (QIIME) software package (Caporaso *et al.* 2010), and in-house Perl scripts were used to analyse alpha and beta diversity. First, the reads were filtered using QIIME quality filters. Then, we used pick_de_novo_otus.py to select operational taxonomic units (OTUs) by creating an OTU table. Sequences with ≥97% similarity were assigned to the same OTUs. We selected a representative sequence for each OTU and with reference to a subset of the SILVA 119 database (http://www.arb-silva.de/download/archive/qiime/) to annotate taxonomic information for each representative sequence. To compute alpha diversity, we rarefied the OTU table and computed two metrics: Richness and Shannon estimated species abundance. QIIME calculates Bray–Curtis, which is a measure of beta diversity.

Statistical analyses

The enzymatic stoichiometric metrics were calculated as described in detail by Moorhead *et al.* (2016). Briefly, the proportional activity of different enzymes was calculated to estimate microbial nutrient acquisition ability. The proportional activity of C- versus N-acquiring enzymes was calculated as $BG/(BG + NAG + LAP)$ corresponding to the *y*-axis on the diagram. The proportional activity of C- versus P-acquiring enzymes was calculated as $BG/(BG + AP)$ corresponding to the *x*-axis on the on the diagram. Metric length refers to the line from the point to the plot origin; it was calculated as $\mathrm{sqrt}(x^2 + y^2)$, and the angle was calculated as degree [ATAN2 (x,y)].

One-way ANOVA was used to test for elevation effects on pH, soil nutrients, enzyme activities and plant root nutrient status, with Tukey's tests to discriminate between elevations ($P \leq 0.05$). These tests were performed using SPSS v. 21 (SPSS Inc.). Principal coordinate analysis (PCoA) based on Bray–Curtis distances was performed to assess the variation in bacterial beta diversity among different elevations. PCoA was conducted by using the vegan package for R 3.2.5 (R Core Team 2016). Random forest analysis was used to examine the predictive importance of bacterial phyla, bacterial beta and alpha diversity, soil nutrients and pH on metric angles and lengths. We conducted these analyses with the randomforest package (Liaw and Wiener 2002) for R 3.2.5 (R Core Team 2016), and we also assessed the significance of both the model and each predictor with the rfutilities (Evans and Murphy 2016) and rfpermute (Archer 2016) packages, respectively.

RESULTS

Variations in soil nutrients and enzyme activities belowground of *A. adenophora* along the elevation gradient

Altitude had a significant influence on soil organic carbon, C:N ratio, pH and C and N content of plant roots ($P < 0.05$). With increasing elevation, the soil organic C content was significantly higher above 2000 m elevation than below 2000 m (Table 1). The pH value at 2200 m elevation was 7.8, which was significantly higher than 6.6 at 2400 m elevation, and both were significantly higher than the values at other elevations (Table 1). The roots of *A. adenophora* at high altitudes (above 2000 m) accumulated more nitrogen but not more carbon (Table 1). The N content in the roots was positively correlated with soil organic C, total N and pH; the C content in the roots was negatively correlated with pH (Supplementary Table S1).

The highest activities of carbon and nitrogen cycling enzymes occurred at 2200 m altitude, while the lowest activities of phosphorus cycling enzymes occurred at that altitude (Table 2). At this altitude, the vector angle was the lowest, but the vector length was the highest (Table 2). Opposite extreme values of both angle and length occurred at an altitude of 1800 m (Table 2). This indicates that phosphorus limitation is critical at 1800 m altitude, while critical carbon limitation occurs at 2200 m altitude.

Shift in the bacterial community along an altitudinal gradient

The predominant bacterial phyla in the soil around *A. adenophora* included Proteobacteria, Acidobacteria, Actinobacteria and Bacteroidetes, and the abundance of different phyla differed across the altitudinal gradient (Fig. 1a; Supplementary Table S2). The bacterial community composition in the 1400–1800 m altitudinal range was clustered, which differed from that in the 2000–2400 m altitudinal range (Fig. 1b).

Table 1: Soil chemical properties and plant root C and N along an elevation gradient

	Elevation gradient (m)					
	1400	1600	1800	2000	2200	2400
SOC (g kg^{-1})	20.6 ± 4.1c	22.1 ± 4.8bc	35.9 ± 1.5abc	46.1 ± 7.2a	44.4 ± 6.2ab	44.1 ± 3.8abc
TN (g kg^{-1})	2.4 ± 0.4a	2.1 ± 0.3a	2.3 ± 0.1a	3.3 ± 0.6a	2.8 ± 0.4a	3.6 ± 0.5a
TP (g kg^{-1})	0.5 ± 0.02a	0.7 ± 0.01a	0.8 ± 0.01a	0.7 ± 0.09a	0.7 ± 0.04a	0.9 ± 0.39a
C:N	9.8 ± 1c	12.2 ± 1bc	18.1 ± 0.3a	16.3 ± 0.8ab	18.4 ± 1.3a	14.5 ± 1.6abc
C:P	107.6 ± 17.1a	84.6 ± 17.3a	121 ± 4.2a	159.7 ± 7.6a	154.4 ± 13.8a	166 ± 46.4a
N:P	11 ± 1.2a	6.8 ± 0.8a	6.7 ± 0.2a	9.8 ± 0.7a	8.4 ± 0.7a	11.9 ± 3.5a
pH	5.8 ± 0.12c	6.0 ± 0.05c	5.7 ± 0.02c	5.8 ± 0.21c	7.8 ± 0.04a	6.6 ± 0.01b
Rt C (%)	38.1 ± 1.4ab	34.8 ± 0.9c	40.3 ± 0.1a	41.1 ± 0.4a	37.8 ± 0.4ab	38.5 ± 0.3a
Rt N (%)	0.8 ± 0.03ab	0.7 ± 0.04c	0.7 ± 0.05c	1 ± 0.06ab	1 ± 0.1a	1.1 ± 0.08a

All data are expressed as the mean ± standard error of the mean (SEM), where n =3. Lowercase letters in the same row denote significant differences ($P \leq 0.05$). SOC, TN and TP refer to soil organic carbon, total nitrogen and total phosphorus, respectively. C:N refers to the ratio between soil organic C and total nitrogen; C:P refers to the ratio between soil organic C and total phosphorus; N:P refers to the ratio between total nitrogen and total phosphorus. Rt C and Rt N refer to root carbon and nitrogen, respectively.

Table 2: Soil enzyme activities and vector dimensions for elevation transects

	Elevation gradient (m)					
Variable	**1400**	**1600**	**1800**	**2000**	**2200**	**2400**
Vector angle	70 ± 1ab	62 ± 2bc	72 ± 3a	66 ± 1abc	51 ± 1d	58 ± 1cd
Vector length	0.5 ± 0.03b	0.6 ± 0.05ab	0.5 ± 0.04b	0.7 ± 0.01ab	0.7 ± 0.03a	0.7 ± 0.02ab
BG (nmol g^{-1} h^{-1})	241 ± 24a	319 ± 67a	201 ± 39a	410 ± 78a	424 ± 80a	299 ± 54a
LAP (nmol g^{-1} h^{-1})	43 ± 12b	47 ± 6b	58 ± 3b	54 ± 11b	119 ± 7a	51 ± 9b
NAG (nmol g^{-1} h^{-1})	188 ± 20a	204 ± 49a	131 ± 20a	219 ± 43a	217 ± 46a	182 ± 22a
AP (nmol g^{-1} h^{-1})	1038 ± 105ab	735 ± 85abc	994 ± 97abc	1113 ± 190a	497 ± 52c	565 ± 106bc

All data are expressed as the mean ± standard error of the mean (SEM), where n =3. Lowercase letters in the same row denote significant differences ($P \leq 0.05$). BG refers to β-glucosidase; LAP refers to leucine aminopeptidase; NAG refers to β-*N*-acetylglucosaminidase; and AP refers to acid (alkaline) phosphatase.

Determinants of the soil enzymatic stoichiometric index

Bacterial community beta diversity (PCoA1) and pH were significantly important contributors to the metric angle and length (Fig. 2). The abundances of Proteobacteria, Acidobacteria and Chloroflexi were important for vector angle, and Proteobacteria and Acidobacteria were important for vector length (Fig. 3). This result suggested that soil pH and bacterial community structure were the critical factors for belowground microbial nutrient uptake during the spread of invasive *A. adenophora* with elevation (Fig. 4).

DISCUSSION

Consistent with the first hypothesis, our data demonstrated that there was a turning point in the nitrogen content of the roots and in the content of organic carbon in the rhizosphere of *A. adenophora* at 2000 m altitude. A possible explanation was the change in vegetation type at the altitude of 2100 m in the Ailao Shan. At higher elevations, the vegetation litter decomposes more slowly and forms a thicker humus layer, resulting in a much higher soil organic C content than at lower elevations. Consistent with our results, the highest storage values of the understorey humus layer of *Pinus sylvestris* were found in the sites at higher

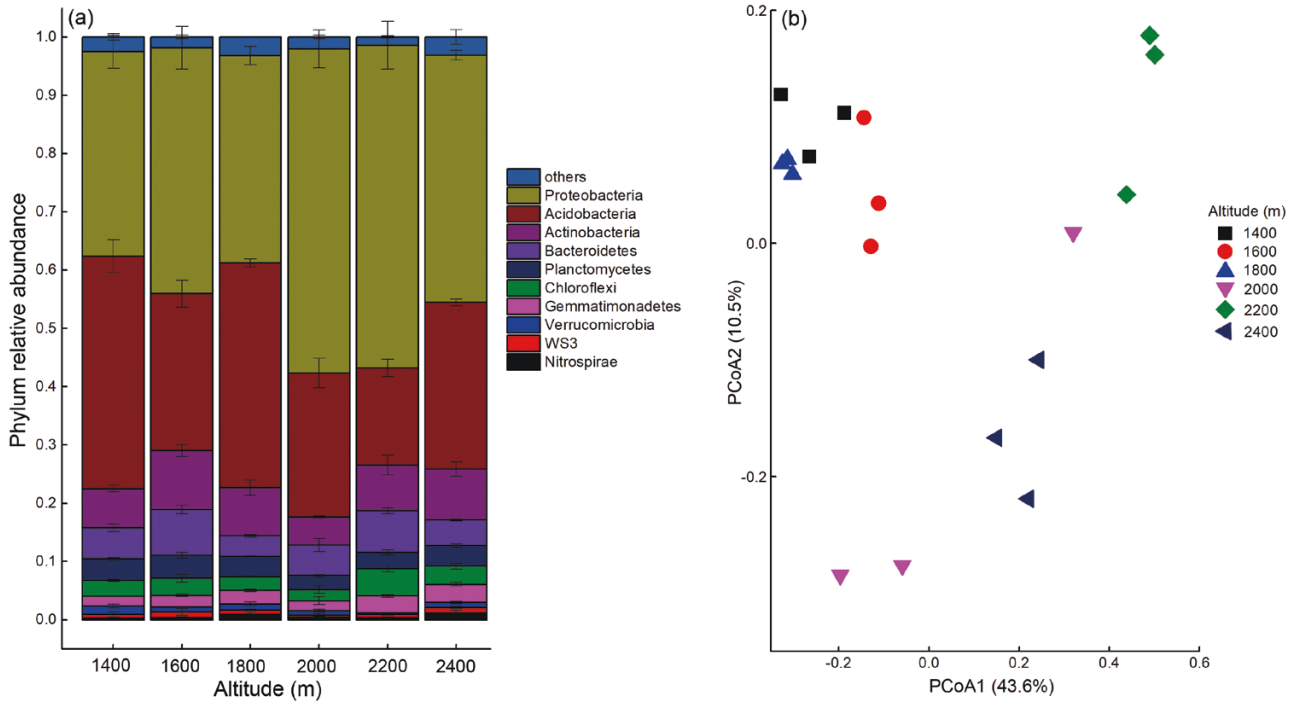

Figure 1: Mean relative abundance of the top 10 phyla (**a**) and sample dissimilarity in the bacterial community (**b**) based on PCoA analysis along elevation transects.

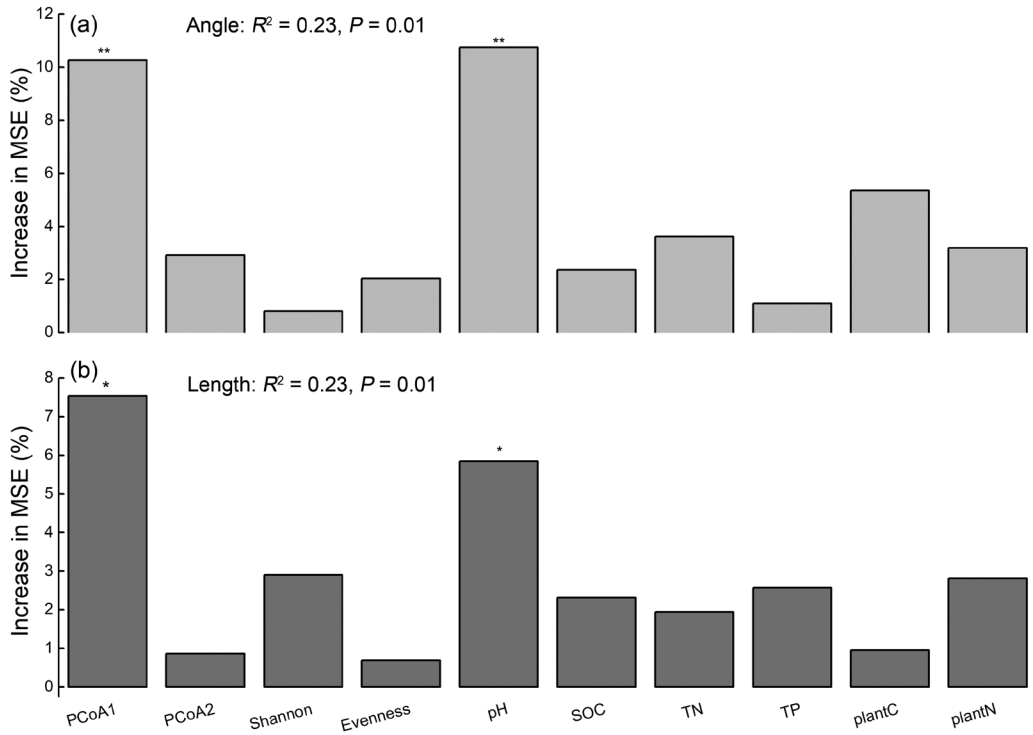

Figure 2: Mean predictor importance (% of increased mean square error) of measured variables on enzymatic stoichiometric vector angle (**a**) and length (**b**) based on random forest analyses. Significance levels of each predictor are indicated by asterisks: *P < 0.05, **P < 0.01. The R-squared value is a measure of how well the model explains the data. The P value for a model determines the significance of the model compared with a null model. Vector angle and length represent phosphorus and carbon limitation for microorganisms, respectively. PCoA1 and PCoA2 represent bacterial beta diversity; Shannon and evenness represent bacterial alpha diversity. Abbreviations: SOC = soil organic carbon, TN = total nitrogen, TP = total phosphorus. Plant C and plant N represent the contents of carbon and nitrogen in plant roots, respectively.

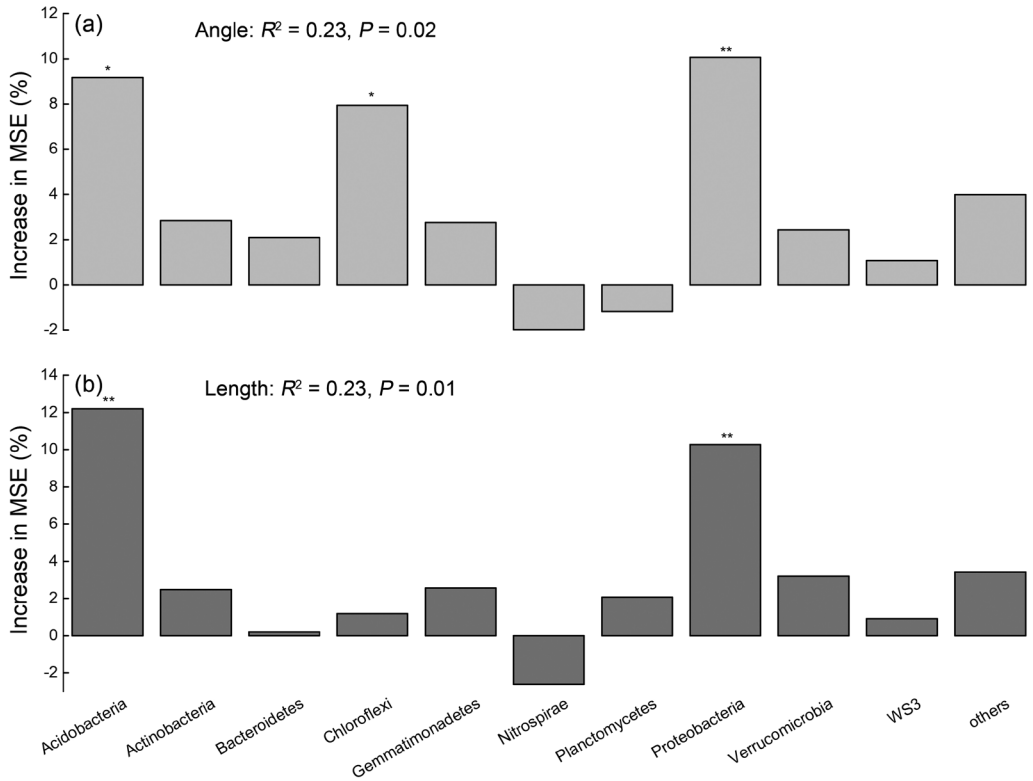

Figure 3: Mean predictor importance (% of increased mean square error) of phyla on enzymatic stoichiometric vector angle (**a**) and length (**b**) based on random forest analyses. Significance levels of each predictor are indicated by asterisks: $*P < 0.05$, $**P < 0.01$. The *R*-squared value is a measure of how well the model explains the data. The *P* value for a model determines the significance of the model compared with a null model. Angle and length represent phosphorus and carbon limitation for microorganisms, respectively.

elevations (Mora *et al.* 2021). In addition, colonization by *A. adenophora* was probably shorter at higher elevations than at lower elevations, which reduced the impact on soil properties. However, the colonization history of *A. adenophora* needs to be determined in the future to decipher the extent of its impact on soil properties.

As the altitude increased, soil organic carbon increased, but the C:N ratio also increased, so the microorganisms invested more energy in synthesizing C-cycling enzymes to decompose the recalcitrant organic matter. This was the reason why soil microbial communities at high altitudes were more constrained by C than by nutrients. The imbalance of elemental stoichiometry in our study area could be a major reason for microbial metabolic limitation due to the homeostatic regulation of microbial biomass elemental composition (Cleveland and Liptzin 2007; Sinsabaugh *et al.* 2009). Another possible reason for C limitation could be related to the increase in abundant microbial populations, which are limited by relative C and P levels and have sensitive metabolic characteristics (Barta *et al.* 2014; Bolscher *et al.* 2017).

Consistent with the second hypothesis, with increasing elevation, the abundance of the phylum Proteobacteria increased significantly, while the phylum Acidobacteria showed a decreasing trend, which contributed to the C limitation in the higher elevation. The phylum Acidobacteria has been consistently associated with oligotrophic environments (Fierer *et al.* 2007), while Proteobacteria are recognized as a phylum associated with copiotrophy (Fierer *et al.* 2012). The ratio of Proteobacteria to Acidobacteria is also used to estimate the trophic status of the soil, with a lower ratio found in oligotrophic environments (Hartman *et al.* 2008). Our results showed that the ratio between the relative abundances of Proteobacteria and Acidobacteria increased from 0.9 at 1400 m elevation to 3.4 at 2200 m elevation (Supplementary Table S1). Thus, we concluded that oligotrophic bacteria dominated at low altitudes, while the abundance of copiotrophic bacteria increased with altitude. More copiotrophic bacteria require more C to sustain their growth, leading to more C-cycling enzyme secretion at high altitudes.

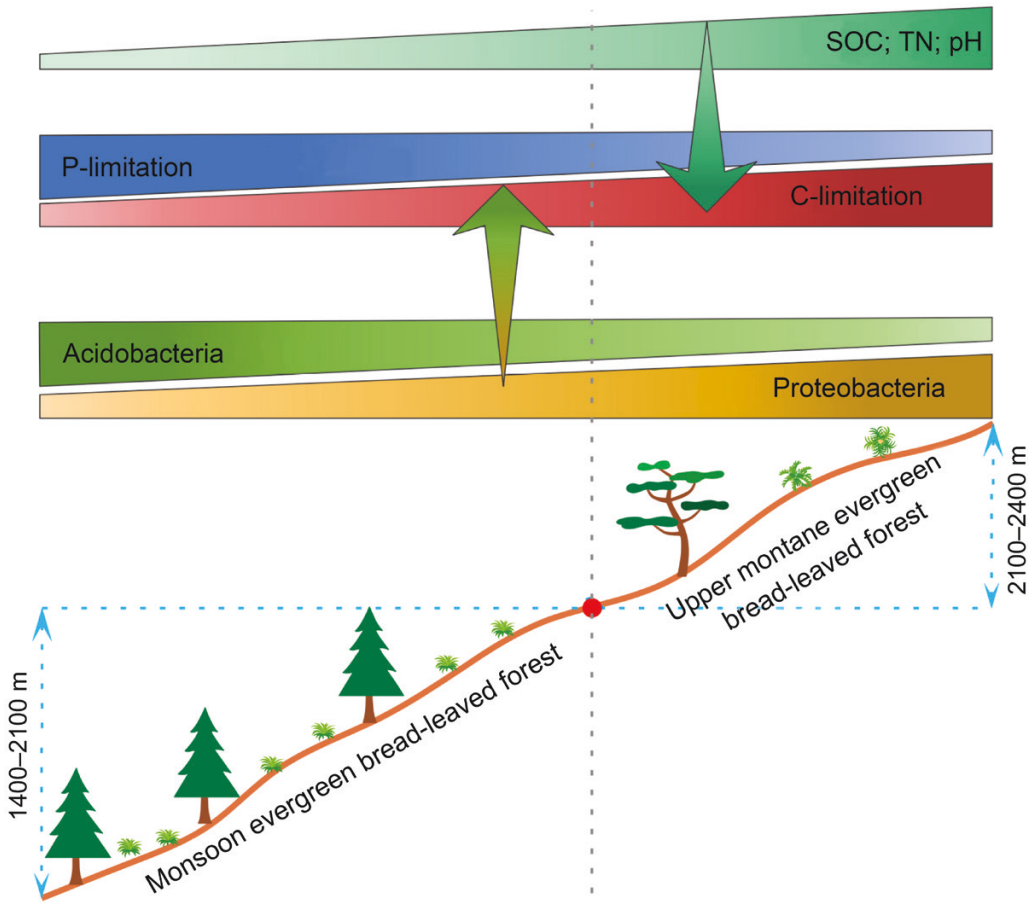

Figure 4: Schematic illustration of the changes in soil chemical and microbiological properties in the rhizosphere of the invader *A. adenophora* along an altitudinal gradient. Abbreviations: SOC = soil organic carbon, TN = total nitrogen.

Phosphorus limitation for microbial growth is ubiquitous across the altitudinal gradient, while at low altitudes, the extent of phosphorus limitation is more intense based on an analysis of enzymatic stoichiometric vector angles (Table 2). It is a widely held view that significant P limitation usually occurs in tropical lowland regions where soils are generally highly weathered (Hou *et al.* 2020; Vitousek *et al.* 2010). Phosphorus limitation in microorganisms leads to high phosphomonoesterase activity in acidic forest soils (Kunito *et al.* 2012). We also found higher P limitation, higher phosphatase activity and lower pH at low elevations. The negative correlation between soil pH and phosphatase activity in different soils is well recognized (Sinsabaugh *et al.* 2008). It was suggested that the ratio of activities for P:N-uptaking enzymes was negatively correlated with relatively low pH in tropical soils (Waring *et al.* 2014), while the opposite relationship was reported in the relatively high pH soils of temperate grasslands (Peng and Wang 2016). Overall, pH differentially affects enzyme activities and thus the enzyme stoichiometric ratio (DeForest and Moorhead 2020; Xu *et al.* 2017).

CONCLUSIONS

The invasive plant *A. adenophora* colonized the Ailao Shan from low to high altitude, showing a significant increase in soil organic carbon, a marked divergence in bacterial community composition and a transition from microbial P limitation to C limitation, with these transitions occurring at 2000 m altitude. Soil pH and bacterial β-diversity were important factors in microbial nutrient limitation; the relative abundance of phyla Proteobacteria and Acidobacteria contributed most to the status of microbial nutrient utilization. The effects of the colonization history of *A. adenophora* on soil properties along the altitudinal gradient need to be further investigated in the future.

Supplementary Material

Supplementary material is available at *Journal of Plant Ecology* online.

Table S1: Spearman's correlation coefficients between plant root C and N and soil chemical properties.
Table S2: Dissimilarity of phyla for elevation transects (mean ± standard error, $N = 3$).

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Conflict of interest statement. The authors declare that they have no conflict of interest.

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