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Large variation in soil phosphorus availability and tree nutritional strategies in humid temperate forests of carbonate rocks

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Abstract (150–250 words)

Background: Carbonate rock soils are typically characterized by high pH and low phosphorus (P) availability, yet these conditions can vary drastically with soil acidification during pedogenesis. Correspondingly, plant nutritional strategies may vary with these soils' changing chemical status. However, empirical data on how the nutritional properties of carbonate rock soils vary with soil acidification and how these variations are linked to plant nutritional strategies remain limited.

Methods: We compared soil chemical properties and P resorption between carbonate and silicate rock forests in two regions differing in soil pH in humid temperate forests. We also measured leaf manganese (Mn) concentration, an indicator of rhizosphere organic acids. Moreover, we explored the influences of soil P availability on plant nutritional strategies for carbonate rock specialists and generalists.

Results: The pH of carbonate rock soils was acidic (4.07–6.58), suggesting advanced weathering under high precipitation. Carbonate rock soils exhibited large variation in total P concentration (220–47,000 mg kg⁻¹), which decreases with decreasing pH, whereas silicate rock soils exhibited smaller variation (300–1,400 mg kg⁻¹). These results suggest that P concentrations in carbonate rock soils peak at the intermediate stage of pedogenesis and decline in later stages. Phosphorus resorption decreased with increasing soil P concentrations regardless of bedrock type. Carbonate rock specialists exhibited remarkably lower resorption efficiency than generalists.

Conclusions: We suggest that soil nutrient availability and plant nutritional strategies in carbonate rock ecosystems vary extensively, and habitat specialization is tightly coupled with plant nutrient-use strategies.

Keywords (4–6)

Calcicole species, Resorption efficiency, Resorption proficiency, Root exudation, Leaf manganese concentration, Walker and Syers' model

Introduction

Carbonate rocks (limestone, dolostone) cover approximately 15% of the earth's ice-free continental area (Ford and Williams 2013). Soils derived from carbonate rocks (carbonate rock soils) often feature high soil pH and high calcium (Ca) concentration, as well as low availability of phosphorus (P) because Ca ions precipitate phosphate at high soil pH (above 6.5) (Lindsay and Moreno 1960). However, limestone soils at relative low pH (5.5 – 6.5) exhibited high soil P availability in tropical Pacific islands (Olsen-P 60–180 mg kg⁻¹) (Schroo 1961; Borggaard et al. 2012) and in a montane deciduous forest of Japan, (Olsen-P, 247.9 mg kg⁻¹) Kajino et al. (2023). The high P availability of these soils surpasses the global mean of bicarbonate-extracted inorganic P (13.2 mg kg⁻¹) (Hou et al. 2018). The high P availability of carbonate rock soils could be explained by P enrichment during rock weathering and subsequent P liberation due to soil acidification. Whitehead et al. (1993) suggested that P is concentrated (total P, up to 71,500 mg kg⁻¹) through intensive leaching of calcium carbonate. As Ca leaching progresses, the accumulated phosphate will gradually solubilize, peaking around pH 6–7 (Schlesinger and Bernhardt 2020) (Figure 1). These findings imply that carbonate rock ecosystems exhibit large variation in P availability. However, this hypothesis relies on observations from limited regions and lacks broad empirical support. Consequently, further empirical studies across broader regions and environmental gradients are required to validate this hypothesis and clarify the variation in P availability in carbonate rock soils.

If carbonate rock ecosystems exhibit a large variation in soil P availability, plant nutritional strategies (acquisition and utilization) might also vary greatly. Leaf nutrient resorption is one of the nutrient utilization strategies in which plants break down nutrient-containing compounds in leaves and absorb them before defoliation, at the expense of energetic costs (Vitousek 1984; Aerts 1996; Kobe et al. 2005; Estiarte et al. 2023). This functional trait slows forest nutrient cycling directly by reducing nutrient flux via litterfall, and indirectly by decreasing litter quality, which retards decomposition rates (Ares and Gleason 2006; Vergutz et al. 2012), thereby regulating the long-term plant-soil feedback during ecosystem development (i.e., pedogenesis + vegetation development). Resorption is expressed in two ways: resorption efficiency, defined as the proportion of nutrients absorbed prior to abscission relative to their concentrations in green leaves; and resorption proficiency, defined as the extent of nutrient reduction during senescence (i.e.,

nutrient concentrations in senesced leaves; (Killingbeck 1996). Previous studies in natural forests demonstrated that plants exhibit higher senesced-leaf P concentrations and lower P resorption efficiency at relatively high P availability. For example, senesced-leaf P concentration increased from 0.54 to 0.97 mg g⁻¹ (Northeastern USA), while P resorption efficiency decreased from 86% to 66% (Malaysia) and from 47% to 30% (China) when comparing sites with low and high P availability (Boerner 1984; Tang et al. 2013; Tsujii et al. 2017). This trend likely reflects a strategy to reduce the energetic expenditure associated with P resorption when environmental P is abundant (Wright and Westoby 2003; Estiarte et al. 2023). A meta-analysis of fertilization experiments has also shown that plants increase senesced-leaf P concentration and decrease P resorption efficiency in environments with high P availability (Yuan and Chen 2015). However, to our knowledge, no studies so far have explored resorption efficiency and proficiency in natural ecosystems with exceptionally high P availability, such as those reported in Schroo (1961) and Kajino et al. (2023). We hypothesized that tree species in environments with extremely high P availability, such as carbonate rock soils at the intermediate pH (Figure 1), show considerably higher senesced-leaf P concentration and lower P resorption efficiency compared to those in environments with normal P availability, such as silicate rock soils.

Rhizosphere effects are defined as the alteration of the physical, chemical, or biological properties of rhizosphere soils by compounds released from plant roots (Phillips and Fahey 2006). The rhizosphere effects are one of plant nutrient-acquisition strategies (e.g. root morphology, capability of organic matter decomposition) and play significant roles in ecosystem nutrient dynamics because they can enhance soil mineral weathering and organic matter decomposition (Marie-Pierre et al. 2009; Finzi et al. 2015). Rhizosphere effects are generally evaluated by directly comparing soil properties between rhizosphere and bulk soils (Phillips and Fahey 2006). The rhizosphere effect on P solubilization by exuding organic acids can be evaluated indirectly by measuring leaf manganese (Mn) concentration (Lambers et al. 2015). Leaf Mn concentration can serve as an indicator of organic acid exudation from roots because organic acids (particularly carboxylates) mobilize not only soil P bound to soil minerals but also Mn (Shane and Lambers 2005; Lambers et al. 2015), and Mn uptake is not specifically regulated due to the lack of Mn-specific transporters, shared with iron and other elements (White and Pongrac 2017; Alejandro et al. 2020). While leaf Mn concentration is a useful tool for estimating organic acid exudation (Lambers et al. 2021; Yan et al. 2024), it should be used with caution when soil Mn availability

differs significantly across sampling locations (White and Neugebauer 2021). Because the rhizosphere effects related to P acquisition (e.g., exudation of organic acids and phosphatases) are generally weak on soils with high P availability (Shane and Lambers 2005; Hayes et al. 2014), we predict that the rhizosphere effect on P solubilization is weak on carbonate rock soils with high P availability. Thus, we hypothesized that rhizosphere effects and leaf Mn concentration decrease with increasing soil P availability.

As carbonate ecosystems are known for their unique plant communities (Shimizu 1959; Yamanaka 1972), species habitat specialization (i.e., low-P specialists, high-P specialists, and generalists) may also be related to P nutritional strategies. Nutritional strategies, including nutrient resorption and rhizosphere effects, differ among species (Hättenschwiler 2008; Wang et al. 2020), and such variations can be attributed to habitat specialization. For example, low-P specialists can genetically exhibit lower tissue and litter P concentration and higher phosphatase activity than generalist species and high-P specialists at a given soil P availability (Gleason et al. 2009; Aoyagi et al. 2025). Several studies reported that specialist species of young carbonate rock soil (calcicole species) can solubilize and absorb P by exudating multivalent organic acids, which possess higher chelating ability. By contrast, calcifuge species (silicate rock specialists) lack this capacity, resulting in significantly lower growth and biomass on carbonate rock soils compared to their performance on silicate rock soils (Ström et al. 1994; Tyler and Ström 1995; Lambers and Oliveira 2019). However, to our knowledge, there are no studies on specialists of developed carbonate rock ecosystems with high P availability. Thus, we hypothesized that calcicole species on carbonate rock soils with high P availability exhibit higher senesced-leaf P concentrations, lower P resorption efficiency, and lower leaf Mn concentrations compared to other species.

In summary, soil chemical status and plant physiological characteristics in developed carbonate rock ecosystems have been studied little; therefore, the long-term biogeochemical dynamics of carbonate rock ecosystems remain unclear. In this study, we investigated soil chemical properties and the nutritional strategies of woody species, including P resorption proficiency/efficiency and rhizosphere effects, across two carbonate rock regions with contrasting levels of soil acidification. We also surveyed silicate rock ecosystems as reference sites in each region. We hypothesized that (1) total P concentration and P availability in carbonate rock soils peak at circumneutral soil pH and then decreases with decreasing pH (H1), (2) plants exhibit higher senesced-leaf P

concentration, lower P resorption efficiency (H2-1), lower leaf Mn concentration (H2-2), and weaker rhizosphere effects on soils with high P availability (H2-3), and (3) these tendencies are more pronounced for calcicole species on carbonate rock soils with high P availability than for other species (H3).

Methods

Study sites

This study was conducted in two sites (Ibuki and Atetsu) with broad-leaved deciduous forests on Japan's Honshu Island (Figure 2). In both sites, carbonate and silicate rocks are adjacently distributed at similar altitudes, and we conducted soil and vegetation surveys in forests on two bedrock types (carbonate rock and silicate rock). Ibuki Mountains (Ibuki; 35°25' N, 136°24'E, highest elevation 1,377m asl) are a mountain range mainly composed of Paleozoic limestone and basalt and Mesozoic sandstone and granite. Mean annual temperature and precipitation (1991–2020) are 9.9 °C and 2,248 mm yr⁻¹. The Atetsu Plateau (Atetsu; 34°56' N 133°32' E, highest elevation approximately 600 m asl) is mainly composed of Paleozoic limestone, sandstone, and basalt. Mean annual temperature and precipitation (1991–2020) are 12.2 °C and 1,378 mm yr⁻¹. We additionally conducted vegetation surveys on Mount Funafuse (Funafuse; 35°39' N, 136°40' E, highest elevation 1,040 m) to verify whether the species' habitat preference (e.g., carbonate rock specialists, silicate rock specialists, and generalists) was consistent. This mountain is mainly composed of Paleozoic limestone and basalt. Mean annual temperature and precipitation (1991–2020) are 10.4 °C and 3,077 mm yr⁻¹. Climate data were obtained from the 1km-Mesh Climate Value 2020 (average over 1991–2020; Japan Meteorological Agency 2022).

Vegetation survey

We conducted vegetation surveys in broad-leaved forests using the Braun-Blanquet approach on 32 plots of carbonate rocks (20 in Ibuki, 9 in Atetsu, and 3 in Funafuse) and 28 plots of silicate rocks (16 in Ibuki, 9 in Atetsu, and 3 in Funafuse; Figure 2). To minimize the effects of local topography, valleys and hollows were avoided when establishing the vegetation survey plots. We set 20 m × 20 m plots and recorded woody plant species and their abundance using six cover classes by visual inspection (“+”, < 1%; “1”, 1–5%; “2”, 5–25%; “3”, 25–50%; “4”, 50–75%; “5”,

75–100%) both for the tree and shrub layers (Poore 1955). The average slope was also recorded. Bedrock types (i.e., carbonate rocks and silicate rocks, including sandstone, granite, and basalt) were identified based on the Seamless Geological Map of Japan (Geological Survey of Japan, AIST 2025) and field inspection of exposed rocks.

Sampling of soils and leaves

Soil cores were collected from 10 plots in Ibuki (carbonate plots = 5, silicate plots = 5) and six plots in Atetsu (carbonate plots = 3, silicate plots = 3). Soil cores were sampled at three depths (0–5 cm, 5–15 cm, and 15–30 cm) using 38 mm diameter core samplers after the litter layers were removed. Although the litter layer is a component of P cycling, its total mass was not quantified due to field survey time constraints. While we acknowledge the exclusion of the litter pool as a limitation of this study, we believe it has minimal impact or bias on discussions regarding P pool and cycle as the litter layers were consistently not thick (generally less than 3 cm). In each plot, soil cores were sampled at five random locations and then combined into one composite for each depth. The soil samples were immediately refrigerated and brought back to the laboratory, and weighed under field-moist conditions to calculate bulk density. The soils were sieved through a 2 mm sieve before chemical analysis. To determine the moisture content of the soils, the subsamples were dried at 70 °C for 72 hours, and water content was determined gravimetrically.

Green and senescent leaves were collected from tree species occurring in the plots where soil core sampling was conducted. Green leaves were collected from near the top of the canopy using a 20 m telescopic carbon pole with a sickle in September 2022 and July–September 2023, whereas senescent leaves were collected in October–November 2022 by shaking tree branches. Green and senescent leaves were collected from three individual trees per species (except for some species, which included one or two individuals) in each plot. The sampled species, number of individuals, and plots are listed in Table S2 (green leaves: 22 species, 17 genera, and 16 families; senescent leaves: 16 species, 13 genera, and 12 families). Leaf samples were washed with ion-exchanged water, dried at 70 °C for 2 days, and milled.

We collected rhizosphere and nearby bulk soils from eight representative tree species, categorized into three distribution characteristics: calcicole, calcifuge, and generalist species. For calcicole species, *Euonymus sieboldianus* and *Viburnum sieboldii* were collected exclusively from carbonate rock plots (six individuals per species). For generalist species, which occur frequently

on both bedrock types, *Acer shirasawanum*, *Tilia japonica*, *Quercus serrata*, and *Pieris japonica* were sampled from both carbonate and silicate rock plots (three individuals per bedrock type per species), except for *P. japonica*, which was collected only from carbonate rock plots (three individuals). For calcifuge species, *Clethra barbinervis* was collected exclusively from silicate rock plots (six individuals). Regarding site distribution, *A. shirasawanum*, *T. japonica*, *E. sieboldianus*, and *V. sieboldii* were sampled only in Ibuki, while *Q. serrata* and *P. japonica* were sampled only in Atetsu. *Clethra barbinervis* was the only species collected from both study sites (three individuals per site). Fine roots and rhizosphere soils for each target species were collected by tracing them from the trunk. Rhizosphere soils were defined as soils adhering to fine roots after gentle shaking (Phillips and Fahey 2006). Bulk soils were defined as soils not adhering to the root surface and are assumed to be less affected by root exudation. The samples were brought back to the laboratory and stored in a refrigerator at 4 °C until chemical analysis. Each soil sample was sieved through a 2 mm sieve before analysis. To determine the moisture content of each soil sample, subsamples were dried at 70 °C for 72 hours, and gravimetric water content was measured.

Chemical analysis

Soil pH was determined from a slurry of 1:2 (w:w) fresh soil to H₂O using a pH meter (D-54SE, HORIBA, Japan). The concentrations of total carbon (C) and total nitrogen (N) in air-dried soils were analyzed by a CHNS-O analyzer (Flash 2000, Thermo Fisher, USA).

Concentrations of P fractions in soils of all sampled depths were measured using the Hedley fractionation method (Tiessen and Moir 1993). Approximately 1 g of fresh soil was sequentially extracted with 0.5 M NaHCO₃ (pH adjusted to 8.5), 0.1 M NaOH, and 1 M HCl (30 mL volume each). We omitted the first step described by Tiessen and Moir (1993), in which an anion-exchange resin was used. We acknowledge that our P sequential extraction method is not rigorously compatible with the standard method, but exclusion of resin extraction does not compromise the comparative evaluation of the primary P fraction and P availability. We determined the inorganic P concentrations in extracts after precipitating organic matter by acidifying subsample solutions with 0.9 M H₂SO₄, following Murphy and Riley's (1962) method. Inorganic P concentrations in the extracts were measured using the molybdenum blue colorimetric method using a spectrophotometer (SH-9000, CORONA, Japan) at 700 nm. Total P concentration in the NaHCO₃ and NaOH extracts was determined with an inductively coupled plasma optical

emission spectrometer (ICPS; ICPS-7510, Shimadzu, Japan). Organic P concentrations in NaHCO_3 and NaOH were calculated as the difference between total P and inorganic P concentrations. Total P concentrations in soils were determined after concentrated H_2SO_4 and H_2O_2 digestion. We continued heating the extracts at 350 °C until the residue became white. The concentration of total P in each digested sample was analyzed by the ICPS. We considered that NaHCO_3 -extracted P is labile P, NaOH-extracted P is moderately labile, Fe/Al-bound P, HCl extracted P is Ca-bound P, and the residual P is occluded P. Estimating P species via sequential extraction method involves uncertainty (Gu et al. 2020; Barrow et al. 2021). For instance, Fe/Al-bound P may be underestimated, while Ca-bound P may be overestimated due to the formation of Ca phosphate precipitates during NaOH extraction. In addition, Ca-bound P can be overestimated through the dissolution of amorphous Fe/Al phases during HCl extraction. However, deeming a broad characterization of P status to be feasible (Yang and Post 2011; Wu et al. 2014), we used the resulting chemical fractions as indicators of dominant phosphorus species in this study. We considered the sum of inorganic P extracted by NaHCO_3 and NaOH as readily available P.

Exchangeable Ca, magnesium (Mg), Mn, and iron (Fe) concentrations in the soil were determined using the ICPS after the extraction of 5 g of fresh soil with 50 ml of 0.5 M barium chloride (Hendershot and Duquette 1986). Soil pH, exchangeable Mn, and soil P fractions (extracted by NaHCO_3 , NaOH, and HCl) of rhizosphere and bulk soils were also measured with the above method.

Leaf Ca, Mg, Mn, and P concentrations were measured using the ICPS after concentrated H_2SO_4 and H_2O_2 digestion. When the precipitant of CaSO_4 appeared, we added concentrated HCl to solubilize it.

Soil P stock estimation and comparison to limestone P stock

Soil P stocks were calculated by multiplying soil bulk density, sampling thickness, and total P concentration for each core. Under the assumption that all P within the top 30 cm of soil originates from carbonate rock, we calculated the equivalent quantity of carbonate rock required to supply this P. This mass balance calculation was based on a carbonate rock density of 2.71 g cm^{-3} (the density of calcite, the primary component of carbonate rock) and a P concentration 2460 mg kg^{-1} (reflecting the maximum P concentration reported in the study area) (Tsukawaki and Ozaki 1953; Igarashi and Fujinuki 1974; our preliminary data).

Statistics

We performed canonical correspondence analysis (CCA)—a multivariate direct gradient analysis that uses chi-squared distance—to determine the habitat preference of each species, using the *cca* function in the "vegan" R package (Dixon 2003). The response variable was woody plant species composition data: the coverage scales of each species were transformed to percentages ("+" to 0.5%, "1" to 2.5%, "2" to 15%, "3" to 37.5%, "4" to 62.5%, and "5" to 87.5%). Explanatory variables were sites (Ibuki, Atetsu, or Funafuse), elevation, slope, and bedrock types (carbonate or silicate). The CCA axis-2 represented the habitat preference associated with bedrock types, as described later (Table 1, Figure 3). Therefore, we used the CCA axis-2 scores of each species to represent their habitat along the soil environment gradient: greater values indicate that the species occur more frequently on carbonate rocks. The significance of each explanatory variable was tested using the *anova.cca* function in the vegan package. The number of permutations was set at 1,000. To assess the CCA axis-2 scores, we compared the scores with established plant strategy frameworks, such as leaf economics spectrum and mycorrhizal association. The full details of methods and results of the comparison are provided in the Supplementary Information.

Analysis of variance (ANOVA) was performed to assess the influence of sites, soil layers, and bedrock types on soil chemical properties (pH, total C, total N, total P, each P fraction, exchangeable Ca, exchangeable Mg, exchangeable Fe, and exchangeable Mn). Linear models were built to evaluate the relationships between soil pH (i.e., an indicator of pedogenetic stages of carbonate rock soils) and P fractions of the carbonate rock soils. Equation 1 shows the model structure, where [P fraction] is each P fraction concentration, [pH] is soil pH, [Depth] is a categorical variable with three levels (0–5 cm, 5–15 cm, and 15–30 cm), and [Site] refers to study sites (Ibuki and Atetsu).

$$[\text{P fraction}] \sim [\text{pH}] + [\text{Depth}] + [\text{Site}] \quad (\text{Equation 1})$$

Phosphorus concentrations of green and senescent leaves were averaged for each species in a plot to calculate P resorption efficiency. We corrected the effect of the leaf mass loss during leaf senescence on P resorption (Van Heerwaarden et al. 2003) by using the mass loss correction factor (MLCF: 0.784 for deciduous broad-leaved species and 0.780 for evergreen broad-leaved species; Vergutz et al. 2012). We calculated P resorption efficiency using Equation 2, where $[\text{P}]_{\text{leaf}}$ refers to the P concentrations of green leaves, and $[\text{P}]_{\text{senescent}}$ refers to the P concentrations of senescent

leaves.

$$\text{P resorption efficiency [\%]} = \left(\frac{[\text{P}]_{\text{green}} - [\text{P}]_{\text{senescent}} \times \text{MLCF}}{[\text{P}]_{\text{green}}} \right) \times 100 \quad (\text{Equation 2})$$

To examine the effects of soil nutrient availability and tree habitat specialization on leaf nutrient concentration and resorption, we employed linear mixed-effects models using the *lmer* function in the R package lme4 (Bates et al. 2003). Equation 3 showed the model structure, where [Nu]_{leaf} refers to leaf Ca, or, Mg, or P concentrations, [Nu]_{soil} is the soil nutrient concentration corresponding to [Nu]_{leaf} (exchangeable Ca or exchangeable Mg or bioavailable P concentration), [CCA2] is the CCA-axis2 score of each tree species. Plant species and ID of plots were included as random intercepts.

$$[\text{Nu}]_{\text{leaf}} \sim [\text{Nu}]_{\text{soil}} + [\text{CCA2}] + [\text{Nu}]_{\text{soil}} \times [\text{CCA2}] + (1 | \text{species}) + (1 | \text{plot})$$

(Equation 3)

To test H2-1 and H3, we built models as follows:

$$[\text{Litter P concentration or P resorption efficiency}] \sim [\text{P}]_{\text{soil}} + [\text{CCA2}] + [\text{P}]_{\text{soil}} \times [\text{CCA2}] + (1 | \text{species}) + (1 | \text{plot})$$

(Equation 4)

In this model, negative P resorption efficiency was treated as 0 %, because negative efficiencies can arise from sample variation or mass loss during leaf senescence, rather than active P accumulation.

To test H2-2 and H3, we built the following model:

$$[\text{Mn}]_{\text{leaf}} \sim [\text{Mn}]_{\text{soil}} + [\text{P}]_{\text{soil}} + \text{CCA2} + [\text{Mn}]_{\text{soil}} \times [\text{P}]_{\text{soil}} + [\text{Mn}]_{\text{soil}} \times [\text{CCA2}] + [\text{P}]_{\text{soil}} \times [\text{CCA2}] + (1 | \text{species}) + (1 | \text{plot})$$

(Equation 5)

where [Mn]_{leaf} is the leaf Mn concentration, [Mn]_{soil} is the concentration of soil exchangeable Mn, and [P]_{soil} is the soil's total P concentration. All variables except for CCA axis-2 and P resorption efficiency were log-transformed to ensure the normality of the residuals, and all variables were standardized.

A paired t-test was conducted to examine rhizosphere effects (i.e., differences in soil pH and exchangeable Mn concentration between bulk and rhizosphere soils). To test H2-3, we also

examined the effect of soil P availability on the magnitude of rhizosphere effects with the following linear mixed models:

$$[\text{pH}]_{\text{rhizo}} - [\text{pH}]_{\text{bulk}} \sim [\text{P}]_{\text{bulk}} + (1 | \text{plot}) \quad (\text{Equation 5})$$

$$[\text{Mn}]_{\text{rhizo}} - [\text{Mn}]_{\text{bulk}} \sim [\text{P}]_{\text{bulk}} + (1 | \text{plot}) \quad (\text{Equation 6})$$

where [pH] is soil pH, [Mn] is the soil's exchangeable Mn concentration, and [P] is bioavailable P. Subscripts, such as "rhizo" and "bulk," indicate the soil properties of rhizosphere and bulk soils, respectively. Plot was included as random intercepts. Species were removed from random intercepts in order to avoid overfitting. The left-hand sides of the equations represent the magnitude of rhizosphere effects.

To confirm the effect of the leaf economics spectrum and mycorrhizal types on nutritional strategies, we built structural equation models. The full details and results of these models are provided in the Supplementary Information.

Results

Vegetation analysis

Overall, 165 woody tree species were recorded across the three sites. In Ibuki, the carbonate plots were mainly dominated by *E. sieboldianus*, *A. shirasawanum*, and *Zelkova serrata*, and the silicate plots were mainly dominated by *A. shirasawanum* and *Quercus crispula*. In Atetsu, *Q. serrata* dominated both bedrock types. In Funafuse, the carbonate plots were mainly dominated by *Q. crispula* and *A. pictum*, and the silicate plots were mainly dominated by *Q. serrata*. Variations in tree species composition across sites, elevations, and bedrocks were analyzed using CCA (Table 1, Figure 3). Site (Ibuki, Atetsu, or Funafuse) and elevation were associated with the first CCA axis, while bedrock type (carbonate or silicate) was associated with the second CCA axis. Species associated with carbonate rocks exhibited greater CCA axis-2 scores (Table S1). Therefore, we used species' CCA axis-2 scores to represent species associations with carbonate rocks in the subsequent analysis. We categorized species at the upper 30th percentile of CCA axis-2 scores as calcicole species, such as *E. sieboldianus*, *V. sieboldii*, and *Syringa reticulata* in Ibuki and *Platycarya strobilacea*, *Morus cathayana*, and *Euonymus oxyphyllus* in Atetsu. Most species

at the lower 70th percentile of CCA axis-2 scores, such as *Q. crispula*, *A. shirasawanum*, and *Zelcova serrata* in Ibuki and *Quercus serrata* and *Z. serrata* in Atetsu, appeared in both the carbonate rock plots and the silicate rock plots, but the species with the three lowest CCA axis-2 scores, such as *Quercus acuta*, *Castanea crenata*, and *C. barbinervis*, appeared exclusively in silicate rock plots. Thus, we categorized species with the three lowest CCA axis-2 scores as calcifuge species, and the others as generalist species.

Soil chemical properties

Soil pH was greater in carbonate rock plots than in silicate rock plots and generally increased with soil depth (Table 2). Soil pH and exchangeable Ca concentrations in carbonate rock plots tended to be higher in Ibuki than in Atetsu, especially in 0–5 cm layers, although the difference between the sites was not statistically significant (Table 2). Concentrations of all P fractions were greater in Ibuki than in Atetsu for both bedrock types (Figure 4). Soil pH in carbonate rock soils was positively correlated with NaOH-extracted inorganic P, HCl-extracted P, and total P (Table 3, Figure 5). By contrast, no significant correlations were observed between soil pH and the NaHCO₃-extracted (inorganic and organic) or NaOH-extracted organic P fractions.

Across all soil layers combined in Ibuki, total P concentration was greater in carbonate rock plots than in silicate rock plots, although one core (15–30 cm) exceptionally showed P concentrations as low as those in the silicate rock plots. Specifically, P availability in the surface layer was 2–17 times higher, and total P concentration was 1.4–60 times higher in carbonate rock plots than in silicate rock plots. The maximum total P concentration in Ibuki carbonate soils reached 47,000 mg kg⁻¹. By contrast, in Atetsu, concentrations of all P fractions in carbonate and silicate rock plots were at the same level. The carbonate soils in Atetsu exhibited a low proportion of HCl-extracted P and a high proportion of residual P (0.36–0.99 %, 50.46–72.87 %, respectively), compared to those in Ibuki (1.06–94.94 %, 0.48–48.63 %, respectively). Across two study sites, both total P and HCl-extracted P concentrations in carbonate rock soils declined sharply with decreasing soil pH (Table 3, Figure 5).

Phosphorus stock and limestone thickness required for P supply

Topsoil (0–30 cm) P stocks in the carbonate plots of Ibuki ranged from 334 to 1,475 g m⁻². In Atetsu, topsoil P stocks were consistently lower than those in Ibuki (Table S3). Phosphorus mass

balance calculations indicated that the formation of a 1 m soil profile in Ibuki requires the chemical dissolution of 1.4 to 49.2 m of parent carbonate rock (Table S3). Depth profiling in Ibuki revealed distinct vertical stratification depending on the P fraction. NaHCO₃-extracted P peaked sharply in the topsoil (0–5 cm) compared to subsurface layers (5–30 cm; Figure S1a). In contrast, other P fractions and total P showed weaker vertical stratification across the soil depth (Figure S1b).

Leaf nutrient concentrations and nutrient resorption

Green leaf and senesced-leaf P concentration increased, and P resorption efficiency tended to decrease with increasing soil P availability and species CCA axis-2 score (Table 4, Figure 6). Species with high CCA axis-2 scores (i.e., calcicole species) tended to exhibit higher senesced-leaf P concentration and lower P resorption efficiency than species with lower CCA axis-2 scores (Table 4, Figure 6). For example, the calcicole species in Ibuki (i.e., high P availability site), such as *E. sieboldianus*, *V. sieboldii*, and *S. reticulata*, showed high senesced-leaf P concentration and low P resorption efficiency (2.43 mg g⁻¹ and 16.6 % on average, respectively) in contrast to those of other species, including generalist and calcifuge species (1.02 mg g⁻¹ and 51.6 % on average, respectively). Calcicole species in Atetsu (i.e., low P availability site; *P. strobilacea*) also tended to exhibit higher senesced-leaf P concentration and lower P resorption efficiency (0.61 mg g⁻¹ and 46.5 % on average, respectively) than other species (0.36 mg g⁻¹ and 68.5 % on average, respectively). The negative values of P resorption efficiency were treated as 0 % for the calculation of average. The interaction terms between soil P availability and species CCA axis-2 were not significant for both senesced-leaf P concentration and P resorption efficiency.

Leaf Ca concentration was also correlated with species CCA axis-2 scores. The interaction term between soil exchangeable Ca and species CCA axis-2 was not significant (Table 4, Figure S2).

Belowground nutritional strategies

Leaf Mn concentration tended to increase with soil exchangeable Mn and decreased with increasing species CCA axis-2 score (Table 4, Figure 7b, c). The interaction term between soil P availability and soil exchangeable Mn was significantly negative, indicating that the effect of soil exchangeable Mn on leaf Mn is weakened under high soil P availability condition (Figure 7c). The interaction term between soil P availability and species CCA axis-2 was significantly positive,

indicating that species with lower CCA axis-2 scores exhibit negative correlation between soil P availability and leaf Mn concentration (Figure 7a).

Soil pH was lower and exchangeable Mn concentration was greater in rhizosphere soils than in bulk soils (Figure S4). The difference in soil pH and exchangeable Mn between bulk and rhizosphere soils showed no correlation with soil P availability (Figure S5).

Discussion

This study demonstrated that soil P availability in humid temperate carbonate rock ecosystems is highly variable, reaching a high level at intermediate soil pH (ca. 6.5) and then declining sharply with decreasing soil pH. These results are consistent with H1 (Figure 1). Across all species on both bedrock types, senesced-leaf P concentration increased, P resorption efficiency decreased, and leaf Mn concentrations decreased as soil P availability increased, thereby supporting H2-1 and H2-2 (Figures 6 and 7). Furthermore, the calcicole species—categorized by our CCA analysis (Figure 3)—displayed unique nutritional strategies. They also exhibited remarkably low P resorption compared with generalist species and calcifuge species, providing partial support for H3 (Figure 6). Overall, our findings suggest that soil P availability and plant nutritional strategies in carbonate rock ecosystems vary extensively depending on soil developmental stages and that the calcicole species may contribute to higher surface soil P availability through the positive feedback (i.e., pumping P from the soil and returning high P litters on the soil's surface).

Variation in soil chemical properties among carbonate rock ecosystems

The soil pH of the carbonate rock soils examined in this study (mean = 5.47) was lower than that of typical carbonate rock ecosystems, such as the limestone grasslands in Öland, Sweden (mean = 7.7–7.9), and Royston, the UK (mean = 6.9), and karst forests in Southwest China (mean = 7.43; (Tyler 1992; Wilson et al. 1995; Du et al. 2011). Multiple factors, including climate, parent material, topography, and vegetation, influence soil pH, climatic factors. Particularly, local water balance (the difference between precipitation and evapotranspiration) is critical for soil pH, as the runoff function as the primary drivers of base cation leaching (Slessarev et al. 2016; Bateman et al. 2019). In this study, the average water balance (2000–2025) in Ibuki (1,550 mm yr⁻¹) and Atetsu (840 mm yr⁻¹) was higher than those of other regions reported higher soil pH (150 mm

yr⁻¹ in Öland, 200 mm yr⁻¹ in Royston, and 430 mm yr⁻¹ in Southwest China; NASA Global Land Data Assimilation System, accessed via the ArcGIS Living Atlas of the World). These results suggest that the relatively low pH observed in this study is a consequence of base cation leaching driven by substantial precipitation and runoff. Soil pH and exchangeable base cation concentrations tended to be lower in carbonate rock soils of Atetsu than those of Ibuki. This suggests that soil weathering is more advanced in Atetsu (Table 2). Atetsu is located on the Kibi Plateau, which was considered to be a peneplain (Sonehara et al. 2020), and has a flatter topography than Ibuki. This topographical setting might promote soil weathering in Atetsu. Soils on flat terrain can persist longer and are, thus, subject to continuous chemical weathering and P depletion. Conversely, soils on steep slopes are subject to the continuous supply of fresh parent materials through surface soil erosion and are chemically “rejuvenated” (Vitousek et al. 2003; Porder and Hilley 2011).

In Ibuki, the site with intermediate soil pH, carbonate rock soils exhibited higher P availability and total P concentration than silicate rock soils. The maximum total P concentration in this study was over 47,000 mg kg⁻¹, far surpassing the highest value (9,630 mg kg⁻¹, $n = 5,275$) reported in a global soil P dataset (He et al. 2021) and comparable to mean total P concentrations reported for highly P-rich carbonate rock soils in tropical Pacific islands (up to 93,300 mg kg⁻¹) (Schroo 1961; Whitehead et al. 1993; Borggaard et al. 2012) and guano-affected soils (40,600 mg kg⁻¹) (Irick et al. 2015). Although we could not directly observe P enrichment phase that likely occurring under alkaline condition, the exceptionally high P concentration in the carbonate rock soils (> 47,000 mg kg⁻¹) strongly suggests P enrichment during rock weathering as no previously reported carbonate rocks in Japan exhibit such high P concentrations (mean = 83–409 mg kg⁻¹, max = 12,600 mg kg⁻¹) (Tsukawaki and Ozaki 1953; Kubo 1971; Igarashi and Fujinuki 1974; Okai 1993; Sakata et al. 2015). In fact, the P concentration of carbonate rock sampled in the highest total P plot was 2460 mg kg⁻¹ (our preliminary data). One might argue that the exceptionally high P concentrations resulted from contamination by animal feces. However, this is unlikely because no visual evidence of feces was observed in our collected samples, and typical fecal P concentrations (< ca. 6,000 mg kg⁻¹) are insufficient to account for such elevated levels (Irick et al. 2015; Le Roux et al. 2020). Furthermore, In the soil with the highest total P, HCl-extracted P accounts for up to 94 % of the total P, implying that the accumulated P is predominantly fixed as Ca phosphate. In P-rich carbonate rock soils on tropical Pacific islands, P predominantly exists in

form of crandallite (Schroo 1961; Whitehead et al. 1993; Borggaard et al. 2012; Vogel et al. 2026). This mineral poorly extracted by both acids and alkalis (Williams et al. 1980), and is supposed to be included in the residual P fraction. Thus, the composition of P fractions in the soils with extremely high total P concentrations in this study might differ from that reported in tropical Pacific islands.

In Atetsu, the site with lower soil pH, there were no differences in the concentrations of all P fractions and total P between bedrock types (Figure 4). The low proportion of HCl-extracted P and high proportion of residual P in the carbonate rock soils (Figure 4) provides additional evidence of advanced weathering in Atetsu (Walker and Syers 1976). In addition, total P and HCl-extracted P (index for P bound to Ca) in the carbonate rock soils decline sharply with decreasing soil pH across two study sites. (Table 3, Figure 5). This suggests that the P status of soils derived from different parent materials converges as soil weathering proceeds (Sáez-Sandino et al. 2025).

Topsoil (0–30 cm) P stocks in the carbonate rock plots of Ibuki formed a large pool (334–1,475g m⁻²), representing a 1.6–7 times higher than the global mean (209.7 g m⁻²) (He et al. 2021a), whereas those of Atetsu were lower than the global mean (Table S3). Phosphorus mass balance estimation suggests that 0.16–5.5 m of carbonate rock must solubilize to form a 1 m soil profile in Ibuki. The highest value (5.5 m carbonate rock for 1 m soil) is plausible given that carbonate rocks are much more susceptible to chemical weathering than silicate rocks (Roy et al. 1999; Karim and Veizer 2000; Fan et al. 2014). However, as this calculation relies on maximum values of bedrock P observed in a plot uniformly, further surveys on variation in rock P concentration across plots are needed for precise estimation (Koike and Matsuda 2003; He et al. 2021b). The inputs of external materials, such as Asian dust could alter P dynamics (Gu et al. 2019). Based on a dust deposition rate of up to 20 g m⁻² (Duce et al. 1991; Uematsu et al. 2003) and a dust P concentration of 1086.6 mg kg⁻¹ (Lawrence and Neff 2009), annual P input via Asian dust is estimated at approximately 2.2 mg m⁻² yr⁻¹. While the effect of dust deposition on the P status of carbonate rock soils is non-negligible, it cannot solely account for the high P availability and total P observed in carbonate rock plots of Ibuki, given that both P availability and total P were higher than those of adjacent silicate rock plots (Figure 4). Furthermore, biological P uplift by vegetation may contribute to the high P availability and total P in the Ibuki carbonate rock area. In Ibuki, NaHCO₃-extracted P fractions peaked sharply in the surface layer (0–5 cm) relative to subsurface

layers (5–30 cm) (Figure S1a), whereas other fractions and total P exhibited weaker vertical stratification (Figure S1b). These observations suggest that while biological P uplift reinforces the P availability in the topsoil, it plays a less prominent role in shaping the distribution of other P fractions and total P.

Plant nutritional strategies

Reflecting the large variation in P availability in carbonate rock soils, tree senesced-leaf P concentrations in carbonate rock ecosystems varied drastically, ranging from 0.19 mg g⁻¹ (*Quercus variabilis* in Atetsu, the low P availability site) to 5.02 mg g⁻¹ (*S. reticulata* in Ibuki, the high P availability site). Phosphorus resorption efficiency in carbonate rock ecosystems also exhibited large variations, ranging from 81.1% (*Q. variabilis* in Atetsu, the low P availability site) to -68.9% (*V. sieboldii* in Ibuki, the high P availability site). A negative value suggests P accumulation in senesced leaves (Milla et al. 2006) or more pronounced mass loss during senescence (Bu et al. 2019), although it could be resulted from sample variation. In the carbonate rock ecosystems of Ibuki, where soil P availability was high, the mean senesced-leaf P concentration was 1.89 mg g⁻¹, a higher value than the upper 10th percentile in the global P concentration in senescent leaves of broad-leaved trees (Vergutz et al. 2012), and comparable to P concentrations in senescent leaves in temperate broad-leaved forests where P fertilization was applied (ca. 0.25–2.5 mg g⁻¹) (Gonzales et al. 2023). Senesced-leaf P concentrations increased, and P resorption efficiency tended to decrease with increasing soil P availability (Table 4, Figure 6a, c). This suggests that plants reduce the energetic costs of the physiological processes of P resorption, including the degradation of nutrient-containing compounds and the transport of the nutrient from the source organs to the sink organs (Wright and Westoby 2003; Estiarte et al. 2023).

Species habitat specialization in carbonate rock ecosystems was significantly related to P utilization strategies. The difference in P resorption efficiency between calcicole species and generalists was less pronounced in Atetsu than in Ibuki, although the interaction between CCA axis-2 score and soil P did not significantly affect senesced-leaf P concentration and P resorption efficiency (Table 4, Figure 6). These results suggest that calcicole species in the high P availability environment exhibit distinct P utilization strategies. The lower P resorption in calcicole species could enhance biological P uptake in carbonate rock ecosystems. By pumping P from deeper

horizons and depositing it at the surface through enriched litter, these species may accelerate P enrichment at the soil surface (Figure S1) (Porder and Chadwick 2009; Sáez-Sandino et al. 2025).

We could not obtain consistent results for relationship between belowground strategies and the P availability gradient across the study sites. (Table 4, Figure 7, Figure S4). Leaf Mn concentration can be used as an indicator of rhizosphere organic acids when bulk soil Mn availability is more or less constant across sampling plots (Lambers et al. 2021; White and Neugebauer 2021). In this study, soil Mn availability varied markedly across sites and bedrock types, therefore we included exchangeable Mn in the linear mixed effect model for leaf Mn concentration. The main effect of soil P availability was not significant, but the interaction term between soil P availability and CCA axis-2 was significantly positive, indicating that soil P availability and leaf Mn concentration are negatively correlated among species with lower CCA axis-2 (Table 4, Figure 7a). This suggests that tree species except for calcicole species reduced root exudation as soil P availability increased. On the other hand, we found that the rhizosphere effect—defined as differences in pH and exchangeable Mn between rhizosphere and bulk soils—was significant, but the magnitude of these differences was not correlated with P availability (Figure S4, S5). The lack of correlation between P availability and rhizosphere effects was inconsistent with the results of leaf Mn concentration. This discrepancy between rhizosphere soil analyses and leaf Mn concentrations is likely attributable to timescale differences. Comparisons between rhizosphere and bulk soils may reflect processes occurring over relatively short timescales, whereas leaf Mn concentrations are assumed to integrate plant–soil interactions over longer timescales.

Leaf Mn concentrations of calcicole species were very low and almost constant along soil total P and exchangeable Mn concentrations (Figure 7). The calcicole species exhibited very low leaf Mn concentrations (0.049 mg g^{-1} on average) compared to generalist and calcifuge species (0.42 mg g^{-1} on average). Other calcicole species in Japan, such as *Nandia domestica*, *Rhamnus japonica*, *Kerria japonica*, and *Betula chichibuensis* also exhibited low leaf Mn concentrations (0.005 mg g^{-1} , 0.012 mg g^{-1} , 0.031 mg g^{-1} , and 0.049 mg g^{-1} , respectively) (Tsuda et al. 1985; Mills et al. 1996). Calcicole species might generally exhibit low leaf Mn concentrations. The low variability in leaf Mn concentration suggests that it is not a good indicator of root organic acid exudation for a calcicole species. There are two potential reasons for the low variability, which are not mutually exclusive: First, calcicole species restrict Mn uptake due to their low

requirements of Mn and potentially other elements that share transporters with Mn, such as Fe and Zn (White and Neugebauer 2021). Second, calcicole species do not exudate much organic acid from fine roots (Zhong et al. 2021). Further study is required to test these hypotheses for the Mn ecophysiology of calcicole species.

Although CCA axis-2 scores (i.e., the habitat specialization indicator) partially covaried with SLA and mycorrhizal association types, SLA and mycorrhizal types had only minor effects on plant nutritional strategies, compared to the effects of CCA axis-2 scores and soil nutrient availability (Figure S3). Both the leaf economics spectrum and mycorrhizal association types are widely recognized as key plant nutritional strategy frameworks (acquisitive vs. conservative) and often related to nutritional strategies (Kazakou et al. 2007; Phillips et al. 2013; Zhang et al. 2018). However, in this study, soil nutrient availability and CCA axis-2 scores had a major influence. The CCA axis-2 scores may largely reflect fundamental differences in plant physiology, such as mineral nutrition (Clarkson 1965; Kinzel 1983). Supporting this, leaf Mn concentration exhibited a strong correlation with the CCA axis-2 scores, suggesting that this axis captures species-specific variation in mineral accumulation distinct from structural leaf economics.

Conclusion

We found that P availability of carbonate rock soils in humid temperate deciduous forests is highly heterogeneous and can be extremely high at intermediate soil pH (i.e., in the middle stage of pedogenesis). This finding suggests that P status of carbonate rock soils under humid climates follows an alternative developmental pathway driven by accelerated Ca leaching. Tree P resorption during leaf senescence exhibited a large variation in response to the wide variation in soil P availability. Carbonate rock specialists exhibited a remarkably higher senesced-leaf P concentration and lower resorption efficiency on high P soils than generalists and silicate rock specialists. Our results suggest that P cycling per unit weight of carbon is fast in carbonate rock ecosystems with high P availability, due to the high P concentration in leaf litter. This biological process might also contribute to high P availability in the surface soils of carbonate rock ecosystems due to the enhanced P redistribution within the surface profile. Our findings on soils and plant nutritional strategies provide novel insights into the biogeochemical nature of carbonate rock ecosystems. Furthermore, these findings may provide guidance for optimizing management

in agriculture and forestry across karst landscapes.

Declarations

Competing interests We declare we have no competing interests.

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844 **Large variation in soil phosphorus availability and tree nutritional strategies in humid**
 845 **temperate forests of carbonate rocks**

846

847 Table 1. Result of canonical correspondence analysis (CCA) examining the effect of
 848 environmental factors on tree species composition. Axis scores of explanatory variables, *p*-values
 849 of each explanatory variable (calculated by permutation test), and explained variance of each axis
 850 are shown.

	CCA axis-1	CCA axis-2	CCA axis-3	CCA axis-4	CCA axis-5	<i>Pseudo- F</i> value	<i>p</i> -value

Bedrock: Carbonate rock	-0.120	0.913	-0.356	0.122	-0.104	2.50	0.001
Site: Atetsu	0.950	0.238	0.133	0.097	0.116	3.82	0.001
Site: Funafuse	-0.062	0.029	-0.152	-0.696	-0.698	1.26	0.276
Elevation	-0.945	0.061	0.315	-0.023	-0.058	2.22	0.001
Slope	-0.034	0.207	-0.100	-0.871	0.432	1.20	0.305
Explained variance (%)	6.13	3.95	3.25	2.09	1.51		

851

852

853 Table 2. Soil chemical properties of carbonate and silicate rocks in Ibuki and Atetsu regions. Values are in means $\pm$ standard deviations ($n = 5$ in Ibuki
854 and $n = 3$ in Atetsu). Analysis of variance (ANOVA) was performed to test differences between the bedrock types, sites, and depths (NS $p > 0.05$, * p
855 < 0.05 , ** $p < 0.01$, *** $p < 0.001$).

Site	Depth (cm)	pH	TC	TN	TP	CO3-Po	CO3-Pi	OH-Po	OH-Pi	Ca-Pi	Ca	Mg	Mn	Fe
(Bedrock)														
<i>Ibuki</i> (Carbonate)	0-5	5.51 $\pm$ 0.57	8.1 $\pm$ 3.5	0.96 $\pm$ 0.43	11661 $\pm$ 20108	60.1 $\pm$ 43.1	56.0 $\pm$ 15.4	395 $\pm$ 263	1012 $\pm$ 241	8413.8 $\pm$ 18391.7	3095 $\pm$ 1919	152.6 $\pm$ 80.7	25.1 $\pm$ 15.5	1.1 $\pm$ 1.1
	5-15	5.47 $\pm$ 0.57	4.9 $\pm$ 1.1	0.75 $\pm$ 0.24	11233 $\pm$ 20114	39.0 $\pm$ 34.5	50.4 $\pm$ 7.7	380 $\pm$ 301	1150 $\pm$ 383	9084.2 $\pm$ 19979.2	2051 $\pm$ 1153	97.5 $\pm$ 55.8	12.2 $\pm$ 7.8	0.5 $\pm$ 0.7
	15-30	5.72 $\pm$ 0.56	2.7 $\pm$ 1.1	0.53 $\pm$ 0.14	10860 $\pm$ 20840	22.8 $\pm$ 23.5	33.1 $\pm$ 4.8	289 $\pm$ 218	810 $\pm$ 261	9012.6 $\pm$ 19906.5	1565 $\pm$ 1386	59.7 $\pm$ 36.7	5.2 $\pm$ 5.3	2.1 $\pm$ 3.8
<i>Ibuki</i> (Silicate)	0-5	4.02 $\pm$ 0.27	12.9 $\pm$ 2.8	1.21 $\pm$ 0.29	969 $\pm$ 275	8.4 $\pm$ 5.1	73.8 $\pm$ 24.1	69 $\pm$ 23	387 $\pm$ 148	46.5 $\pm$ 88.3	333 $\pm$ 326	45.5 $\pm$ 30.0	23.8 $\pm$ 9.9	8.0 $\pm$ 1.7
	5-15	4.28 $\pm$ 0.08	8.4 $\pm$ 3.7	0.59 $\pm$ 0.15	822 $\pm$ 275	6.2 $\pm$ 2.7	57.7 $\pm$ 20.1	52 $\pm$ 21	363 $\pm$ 185	39.6 $\pm$ 81.2	99 $\pm$ 117	15.6 $\pm$ 10.7	8.3 $\pm$ 4.1	3.8 $\pm$ 2.0
	15-30	4.58 $\pm$ 0.14	5.6 $\pm$ 3.4	0.60 $\pm$ 0.18	638 $\pm$ 268	3.8 $\pm$ 1.5	34.3 $\pm$ 19.1	45 $\pm$ 35	284 $\pm$ 166	13.7 $\pm$ 26.0	61 $\pm$ 71	11.2 $\pm$ 8.9	5.4 $\pm$ 3.2	2.0 $\pm$ 2.5
<i>Atetsu</i> (Carbonate)	0-5	4.76 $\pm$ 0.60	5.7 $\pm$ 0.6	0.33 $\pm$ 0.07	299 $\pm$ 94	8.0 $\pm$ 1.4	9.8 $\pm$ 1.0	20 $\pm$ 6	93 $\pm$ 33	2.3 $\pm$ 0.5	1683 $\pm$ 644	62.4 $\pm$ 15.7	39.7 $\pm$ 15.6	5.2 $\pm$ 4.5
	5-15	5.36 $\pm$ 0.39	2.7 $\pm$ 0.8	0.20 $\pm$ 0.07	252 $\pm$ 87	4.1 $\pm$ 0.4	3.7 $\pm$ 1.8	13 $\pm$ 6	73 $\pm$ 15	1.3 $\pm$ 0.5	1552 $\pm$ 908	43.0 $\pm$ 19.8	24.5 $\pm$ 16.8	1.8 $\pm$ 3.0
	15-30	5.77 $\pm$ 0.09	1.8 $\pm$ 0.7	0.17 $\pm$ 0.06	200 $\pm$ 57	2.5 $\pm$ 0.4	4.1 $\pm$ 3.1	9 $\pm$ 3	40 $\pm$ 17	1.0 $\pm$ 0.2	1972 $\pm$ 590	37.7 $\pm$ 10.9	19.5 $\pm$ 13.5	0.6 $\pm$ 0.6
<i>Atetsu</i> (Silicate)	0-5	4.38 $\pm$ 0.43	10.2 $\pm$ 3.8	0.52 $\pm$ 0.21	326 $\pm$ 40	7.9 $\pm$ 1.1	16.0 $\pm$ 4.7	22 $\pm$ 4	83 $\pm$ 37	3.1 $\pm$ 2.9	742 $\pm$ 374	151.1 $\pm$ 85.5	63.4 $\pm$ 22.4	3.9 $\pm$ 0.5
	5-15	4.53 $\pm$ 0.27	3.4 $\pm$ 0.6	0.24 $\pm$ 0.05	224 $\pm$ 80	2.6 $\pm$ 0.5	4.9 $\pm$ 4.0	17 $\pm$ 4	43 $\pm$ 11	0.7 $\pm$ 0.7	174 $\pm$ 171	65.9 $\pm$ 77.5	14.9 $\pm$ 10.9	4.2 $\pm$ 4.3
	15-30	4.65 $\pm$ 0.09	2.2 $\pm$ 0.6	0.19 $\pm$ 0.05	214 $\pm$ 101	3.1 $\pm$ 0.9	5.6 $\pm$ 4.3	11 $\pm$ 3	39 $\pm$ 10	1.3 $\pm$ 0.7	137 $\pm$ 146	66.2 $\pm$ 86.9	13.6 $\pm$ 7.9	1.2 $\pm$ 1.2
ANOVA results														
Bedrock		***	***	NS	*	NS	**	***	***	0.08	***	0.06	NS	**
Site		NS	***	***	0.08	***	**	***	***	NS	NS	NS	***	NS
Depth		**	***	***	NS	***	NS	NS	NS	NS	NS	*	***	*

856 Abbreviations: Depth, the depth of soil cores (cm); pH, soil pH; TC, total carbon concentration (%); TN, total nitrogen concentration (%); TP, total
857 phosphorus concentration (mg kg^{-1}); $\text{CO}_3\text{-Po}$, organic phosphorus concentration extracted by NaHCO_3 (mg kg^{-1}); $\text{CO}_3\text{-Pi}$, inorganic phosphorus
858 concentration extracted by NaHCO_3 (mg kg^{-1}); OH-Po , organic phosphorus concentration extracted by NaOH (mg kg^{-1}); OH-Pi , inorganic
859 phosphorus concentration extracted by NaOH (mg kg^{-1}); Ca-Pi , inorganic phosphorus concentration extracted by HCl (mg kg^{-1}); Ca, exchangeable
860 calcium concentration (mg kg^{-1}); Mg, exchangeable magnesium concentration (mg kg^{-1}); Fe, exchangeable iron concentration (mg kg^{-1}); Mn,
861 exchangeable manganese concentration (mg kg^{-1}).

862

863 Table 3. Summary of linear regression analyses examining the effects of soil pH on each P fraction in carbonate rock soils. *R* is the adjusted
864 coefficient of determination of each model. NS, $p > 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

P fraction	CO ₃ -Pi			OH-Pi			Ca-Pi			CO ₃ -Po			OH-Po			TP		
Variable	Coef.	SE	<i>p</i>	Coef.	SE	<i>p</i>	Coef.	SE	<i>p</i>	Coef.	SE	<i>p</i>	Coef.	SE	<i>p</i>	Coef.	SE	<i>p</i>
Intercept	0.83	1.47	NS	0.38	1.05	NS	-12.45	3.76	**	3.58	1.05	**	4.32	0.77	***	-1.02	1.96	NS
Depth: 5-15	-0.64	0.34	NS	-0.32	0.24	NS	-0.86	0.87	NS	-0.40	0.24	NS	-0.02	0.18	NS	-0.42	0.46	NS
Depth: 15-30	-1.26	0.37	**	-0.75	0.26	**	-1.80	0.94	NS	-0.60	0.26	*	-0.46	0.19	*	-1.11	0.49	*
Site: Ibuki	1.80	0.29	***	3.02	0.21	***	4.22	0.75	***	2.38	0.21	***	2.72	0.15	***	2.42	0.39	***
pH	0.24	0.28	NS	0.47	0.20	*	2.69	0.73	**	-0.33	0.20	NS	0.00	0.15	NS	1.32	0.38	**
<i>R</i> ²	0.69			0.92			0.71			0.86			0.94			0.73		

865 Abbreviations: pH, soil pH; TP, total phosphorus concentration (mg kg⁻¹); CO₃-Po, organic phosphorus concentration extracted by NaHCO₃ (mg
866 kg⁻¹); CO₃-Pi, inorganic phosphorus concentration extracted by NaHCO₃ (mg kg⁻¹); OH-Po, organic phosphorus concentration extracted by NaOH
867 (mg kg⁻¹); OH-Pi, inorganic phosphorus concentration extracted by NaOH (mg kg⁻¹); Ca-Pi, inorganic phosphorus concentration extracted by HCl
868 (mg kg⁻¹)

Table 4. Summary of linear mixed-effects models predicting leaf traits based on soil chemical properties and the CCA axis-2 scores of each tree species. R^2_m and R^2_c are marginal and conditional coefficients of determination, respectively. R^2_m represents the variance explained by fixed effects, and R^2_c represents the variance explained by both fixed and random effects. NS, $p > 0.1$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Model	Variable	Coef.	SE	p
Leaf Ca ($R^2_m = 0.46$, $R^2_c = 0.91$)	(Intercept)	-0.06	0.16	NS
	Ca	0.53	0.13	**
	CCA2	0.25	0.11	*
	Ca×CCA2	0.05	0.05	NS
Leaf Mg ($R^2_m = 0.13$, $R^2_c = 0.75$)	(Intercept)	0.00	0.18	NS
	Mg	0.26	0.08	**
	CCA2	0.18	0.16	NS
	Mg×CCA2	-0.02	0.07	NS
Leaf P ($R^2_m = 0.51$, $R^2_c = 0.84$)	(Intercept)	-0.01	0.13	NS
	AP	0.68	0.08	***
	CCA2	0.06	0.10	NS
	AP×CCA2	-0.11	0.06	0.088
Leaf Mn ($R^2_m = 0.33$, $R^2_c = 0.91$)	(Intercept)	-0.17	0.18	NS
	Mn	0.24	0.12	0.08
	AP	-0.25	0.14	NS
	CCA2	-0.47	0.15	**
	Mn×AP	-0.11	0.05	0.056
	Mn×CCA2	0.12	0.08	NS
	AP×CCA2	0.26	0.10	**
Senesced-Leaf P ($R^2_m = 0.52$, $R^2_c = 0.89$)	(Intercept)	-0.01	0.15	NS
	AP	0.60	0.11	***
	CCA2	0.22	0.10	0.058
	AP×CCA2	-0.09	0.06	NS
P Resorption Efficiency ($R^2_m = 0.31$, $R^2_c = 0.71$)	(Intercept)	0.09	0.20	NS
	AP	-0.30	0.17	0.098
	CCA2	-0.34	0.16	*
	AP×CCA2	-0.06	0.13	NS

Abbreviation: AP, soil phosphorus availability (i.e. sum of the NaHCO₃-extracted and NaOH-extracted inorganic P concentration); Ca, soil exchangeable calcium concentration; Mg, soil exchangeable magnesium concentration; Mn, soil exchangeable manganese concentration; CCA2, species CCA axis-2 score; SE, standard error.

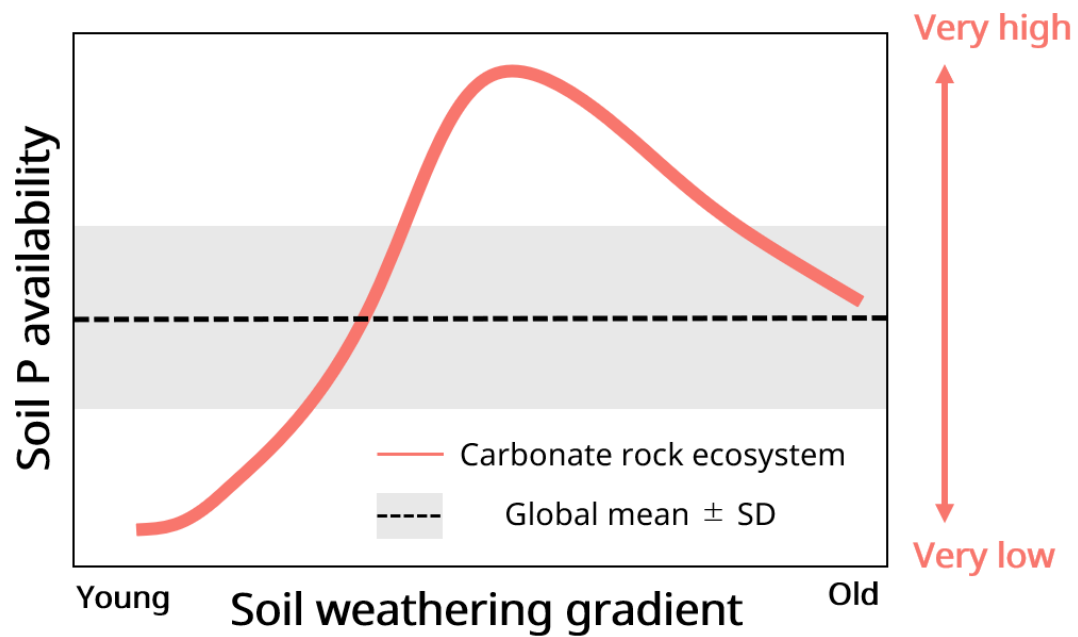


Figure 1. Conceptual diagram illustrating soil P availability along the soil weathering gradient in carbonate rock ecosystems. The red line indicates the trajectory of soil P availability in carbonate rock ecosystems. The horizontal dashed line and gray shaded area indicate the global average and standard deviation across diverse parent materials, respectively.

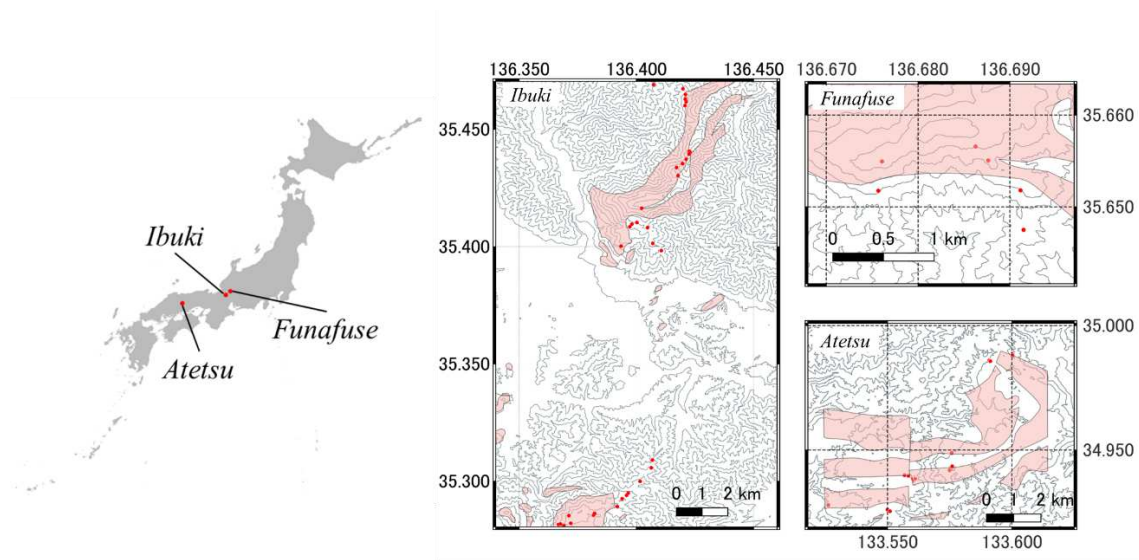


Figure 2. The locations and geological map of research sites. The pink areas represent the distribution of carbonate rock, and the white areas represent the distribution of silicate rock, (Geological Survey of Japan, AIST 2025). Vegetation surveys were conducted at the red points.

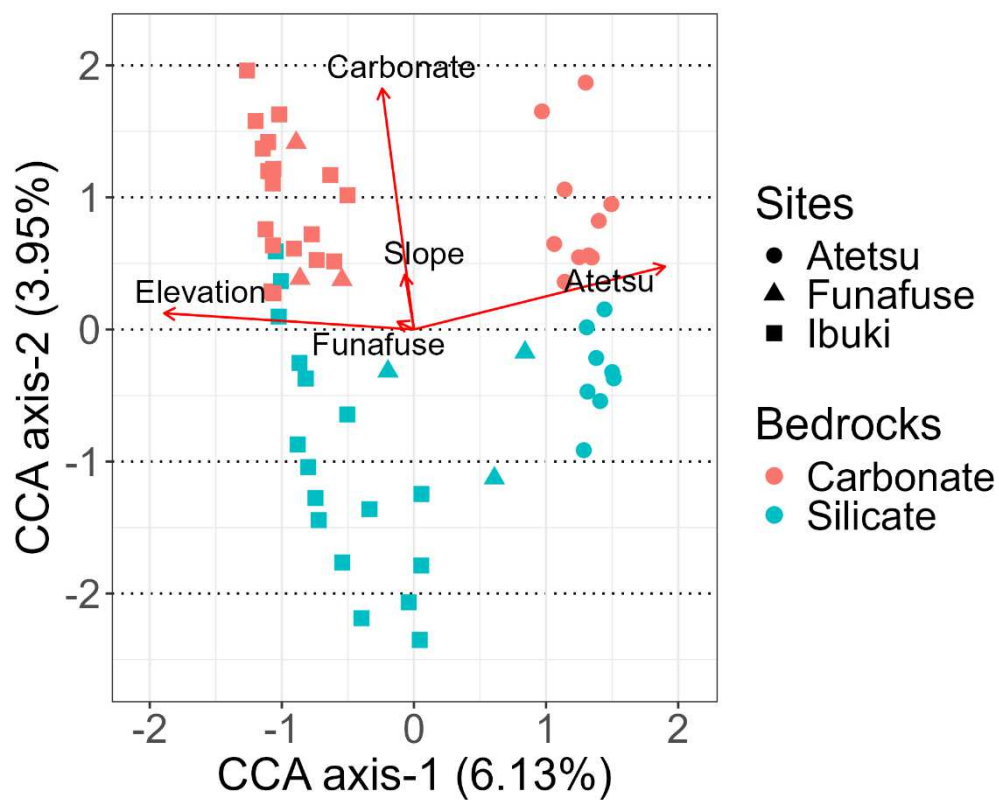


Figure 3. A biplot of canonical correspondence analysis (CCA) of plant communities at carbonate rock and silicate rock plots at three locations in Japan ($p = 0.001$, permutation test by the *anova.cca* function). Each point represents a surveyed plot. Percentages in parentheses indicate the variance explained by the axis. Arrows indicate the contribution of each explanatory variable for axis-1 and axis-2.

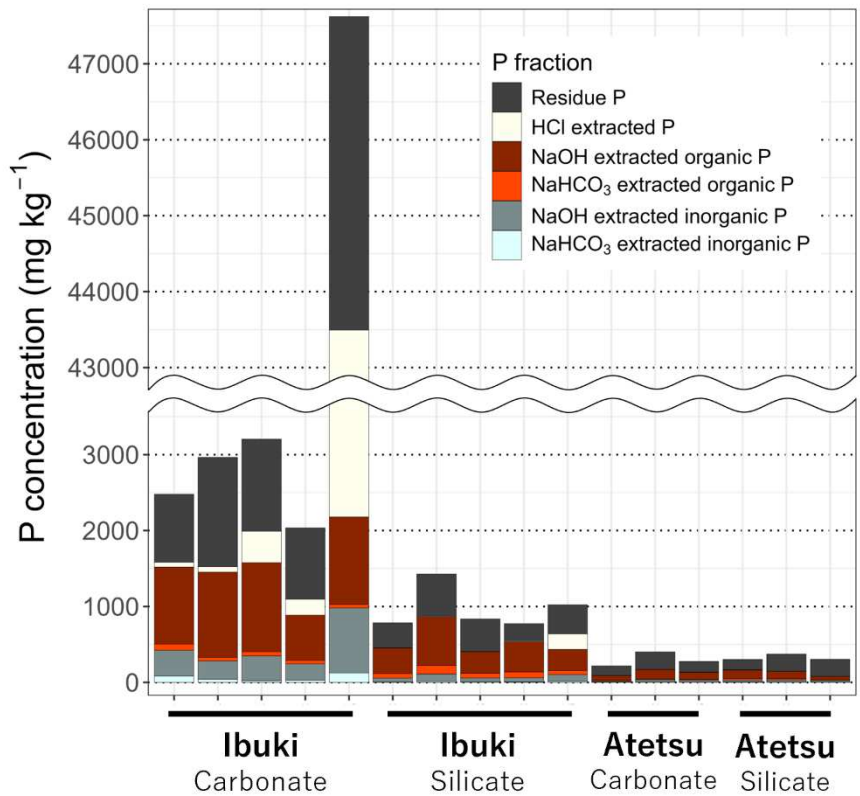


Figure 4. Concentrations of phosphorus (P) fractions in surface soils (0–5 cm) compared between carbonate and silicate bedrocks. Each bar represents a single plot. Fractionation was conducted following the method of Hedley P fractionation (Tiessen and Moir 1993).

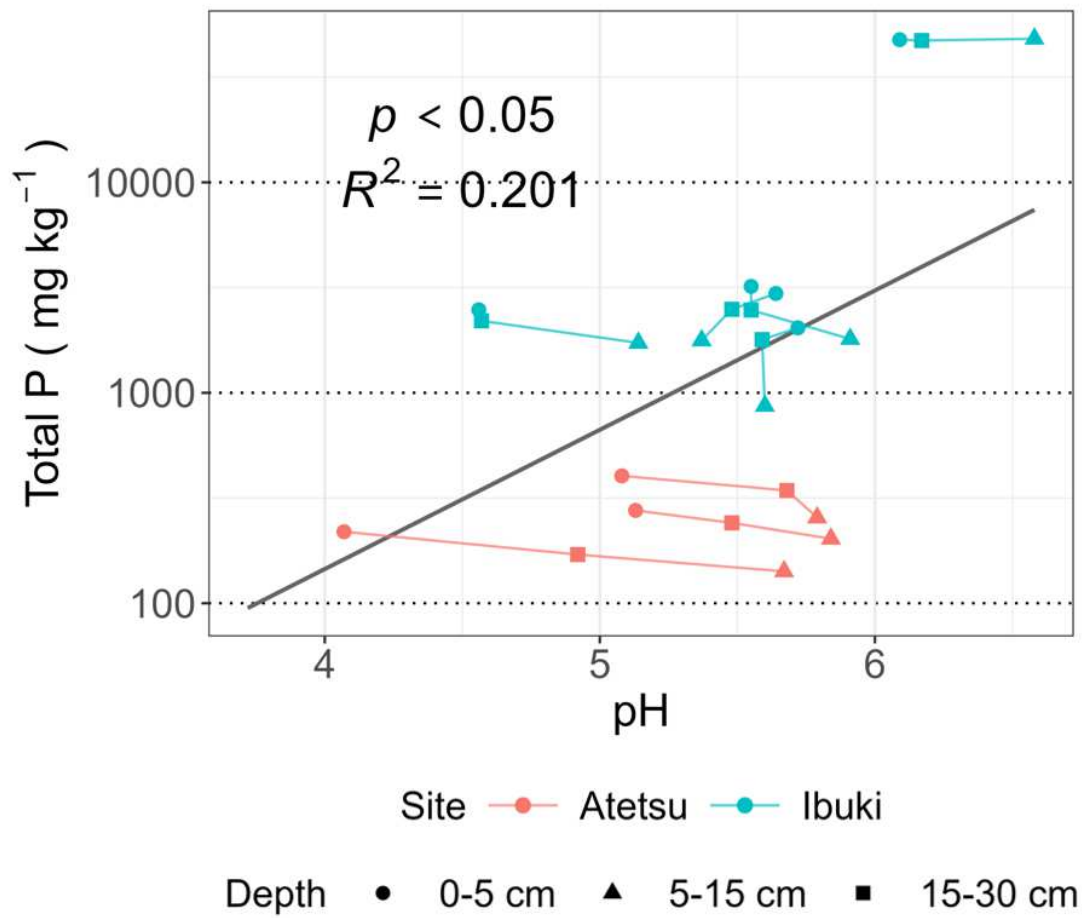


Figure 5. Total P concentration against pH of carbonate rock soils. Each point represents a soil core at a given depth. Points connected by colored lines indicate that they were sampled in the same plot. Black lines were plotted by single linear regression. R^2 and p values were also shown. See Table 3 for the result of multiple regression analysis, including the effects of site, depth, and soil pH.

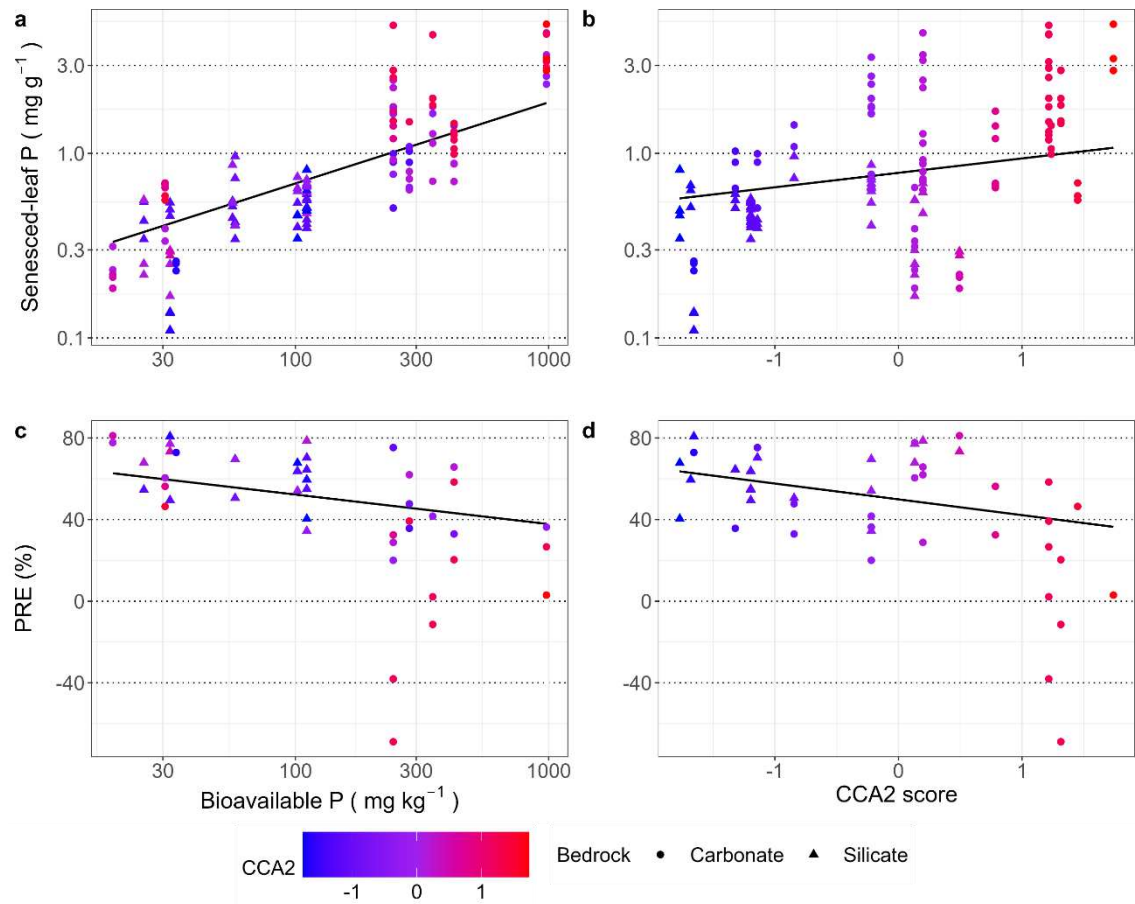


Figure 6. Senesced-leaf P concentration and P resorption efficiency (PRE) in relation to soil phosphorus availability and an index of species habitat associations related to carbonate rocks (the score of the CCA axis-2; Figure 1). Each point represents an individual tree (a, b) and a species mean within each plot (c, d). Soil total P and senesced leaf P concentration were log-transformed. Black lines indicate predictions from linear mixed-effects models, with other covariates fixed at their mean values. Detailed model results, including interaction terms, are provided in Table 4.

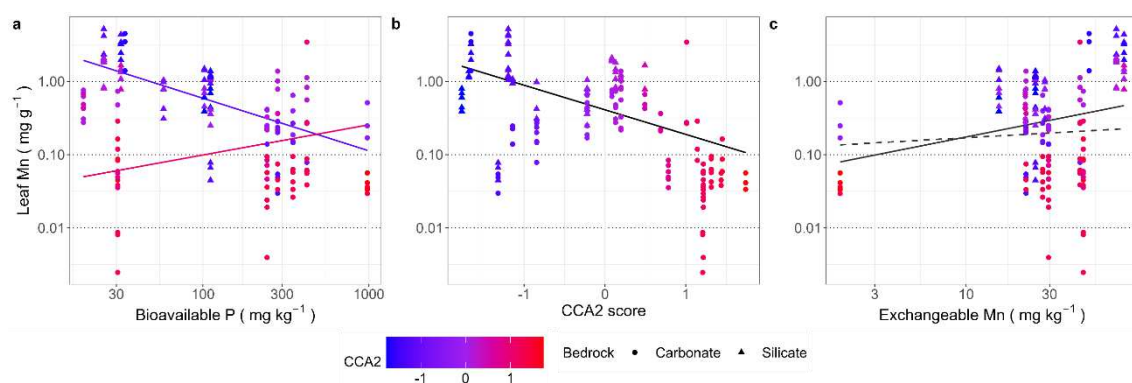


Figure 7. Leaf manganese (Mn) concentration against soil phosphorus availability (a), an index of species habitat associations related to carbonate rocks (the score of the CCA axis-2; Figure 1) (b), and soil exchangeable manganese concentration (c). Each point represents an individual tree. Leaf Mn, soil total P and exchangeable Mn were log-transformed. The red and blue lines in (a) indicate predictions from linear mixed-effects models, with the CCA axis 2 scores held at the 1st and 3rd quartiles, respectively, and other covariates fixed at their mean values. The black line in (b) indicates a prediction from linear mixed-effects models, with all other covariates fixed at their mean values. The black solid and dashed lines indicate that predictions from linear mixed-effects models, with the soil P availability held at the 1st and 3rd quartiles, respectively, and other covariates fixed at their mean values. Detailed model results, including interaction terms, are provided in Table 4.

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