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Variations in soil nutrient availabilities and foliar nutrient concentrations of trees between temperate monsoon karst and non-karst forest ecosystems on Mount Ibuki in Japan

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Abstract

Plants growing on karst soils, which are characterized by high pH (> 7.5-8.0) and low phosphorus availability, often exhibit phosphorus deficiency. However, little is known about the soil nutrient availabilities and foliar nutrient concentrations of trees in karst ecosystems with lower soil pH (< 7.0). In this study, we analyzed soil properties and nutrient concentrations of leaf litter from two secondary forests in the Asian monsoon temperate region of Japan, one on karst (limestone) soil and the other on non-karst (sandstone) soil. We also compared the live leaf nutrient concentrations of four dominant tree species (*Carpinus tschonoskii, Cornus macrophylla, Neolitsea sericea*, and *Quercus variabilis*) found in both sites. The karst soil had a higher pH (6.5) than the non-karst soil (5.6), as well as higher phosphorus concentrations and higher calcium availability, but lower potassium availability. The phosphorus concentrations measured using Truog ($(NH_4)_2SO_4$) and Olsen ($NaHCO_3$) extraction methods were both higher in the karst soil. The availabilities of ammonium and nitrate in the soil did not differ significantly between the sites. The concentrations of calcium, potassium, and phosphorus in the live leaves and leaf litter reflected their availability in the soil, and the litter nitrogen concentration was higher in the karst forest. Overall, this karst soil with a relatively low pH (6.5) was rich in phosphorus but poor in potassium. Karst soil may provide a large quantity of phosphorus for trees at low pH. Future research should investigate the change in phosphorus availability of karst soils at different degrees of weathering.

Keywords: foliar nutrient concentration, karst ecosystem, limestone soil, potassium deficiency, soil phosphorus availability

Introduction

Soil nutrient availabilities significantly influence the characteristics of vegetation. Karst ecosystems, which develop on weathered calcium (Ca)-rich rocks such as dolomite, limestone, and gypsum, are distributed sporadically on Earth and cover more than 10% of the ice-free continental surface (Ford and Williams 2013, Geekiyanage et al. 2019). Karst soils are known for their high soil pH (e.g., 8.1 in Wilson et al. 1995, and 7.7 in Liu et al. 2018), high Ca concentrations (Wei et al. 2018, Tang et al. 2021), and low phosphorus (P) availabilities (Niinemets and Kull 2005, Liu et al. 2014) because Ca ions deposit phosphate at high soil pH (the solubility of $H_2PO_4^-$ steeply decreases with pH in the range of pH of 6.5 and 8.0 under a presence of Ca ion, Lindsay and Moreno 1960). The productivities of ecosystems on calcareous soils, including grasslands and forests, are often under P limitation (Jeffery and Pigott 1973, Wilson et al. 1995, Niklaus et al. 1998, Stöcklin and Körner 1999, Du et al. 2011; Liu et al. 2014, Hu et al. 2022) or co-limited by nitrogen (N) and P (Morecoft et al. 1994, Kooijman et al. 1998, Niinemets and Kull 2005, Du et al. 2011) possibly due to the slow N mineralization rate caused by low P availability (Niinemets and Kull 2005). Plants in karst ecosystems generally have low foliar P concentrations, reflecting low soil P availability (Du et al. 2011, Liu et al. 2014), and thus, P cycling through vegetation in karst ecosystems is generally slow (Zhu et al. 2021).

While previous studies have found that phosphate is deposited by Ca ion and becomes less available for plants in soil with pH of higher than 7.5 (Lindsay and Moreno 1960, Hinsinger 2001), we have less knowledge about how P in the karst soils is released with long-term weathering. Even though availabilities of P in karst soils are generally low, total P concentrations in most karst soils (often higher than 1000 mg g^{-1}, Table S1) are much higher than most soils in the global data (He et al. 2021, 430 mg kg^{-1} in median). As the concentration of calcium phosphate decreases with time in both calcareous (Ryan and Zghard 1980) and non-calcareous soils (Syes and Walker 1969) and eventually becomes zero (Walker and Syes 1976), the large amounts of soil P in karst soils are likely to be released through long-term weathering and decalcification. After decalcification, the pH of limestone soils become less than 7.0, and P deficiency is weakened (e.g., Kooijman et al. 1998, 2021, Ueno 2013). However, we have less knowledge about the P availabilities in karst soils during decalcification. We suspect that the phosphate in karst soils might be released very rapidly when soil pH decreases because the solubility of calcium phosphate increases at lower pH (Lindsay and Moreno 1960). Specifically, we speculated that soil P availability of karst soils substantially increases in the middle of soil weathering and decalcification, before losing their calcium phosphate (Fig. 1). This study hypothesized that karst soils with relatively low pH (approximately between 6.0 and 7.0) but still rich in Ca have high P availabilities. Furthermore, if P availability is high, the N cycling process, such as mineralization or nitrification, may become faster (DeForest and Otuya 2020, Sasaki et al. 2022). Thus, we also hypothesized that karst soils with low pH but still rich in Ca also have high N availability, although low soil pH generally results in a low soil nitrification rate (Ste-Marie and Paré 1999).

In addition to N and P, low potassium (K) availability and co-limitation of N, P, and K have been observed in tropical karst ecosystems in southwest China (Liu et al. 2014, Liu et al. 2018, Zhu et al. 2021), reflecting the low K concentrations of limestones. However, it is still unknown whether these K-deficient conditions are a general characteristic of karst ecosystems because most studies on macroecology have not considered the effect of K availability and deficiency (Sardans and Penuelas 2015).

Sardans and Penuelas (2015) found that up to 70% of terrestrial ecosystems were under K limitation to some extent by reviewing the published data, highlighting the importance of considering K availability in macroecological studies. Therefore, this study also examined K availability in a karst ecosystem in Japan to improve our understanding of nutrient availabilities in karst ecosystems and the distribution of K-deficient ecosystems in the world.

In this study, we investigated the characteristics of a karst forest with a relatively low soil pH (6.5) but still rich in Ca on Mt. Ibuki, Japan. We compared two secondary forests established on limestone and non-limestone (sandstone) soils on Mt. Ibuki, focusing on nutrient availability and cycling. We measured the availability of four elements (Ca, K, N, and P) in the soil. For P, we measured two different fractions in the soil: an alkaline-soluble fraction bound to aluminum (Al) and iron (Fe) ions, and an acid-soluble fraction bound to Ca ions (Olsen-P and Truog-P, respectively, see methods). We also measured the concentrations of the four elements in the leaves of four common species (*Carpinus tschonoskii*, *Cornus macrophylla*, *Neolitsea sericea*, and *Quercus variabilis*) across the sites. In addition to fresh leaves, we quantified element concentrations in leaf litter on the forest floor because the nutrient concentrations of leaf litter reflect element availabilities in the soil more clearly than live leaves (Vitousek 1982). We predicted that the P availability of this karst soil would be high because the solubility of calcium phosphate would be high at its relatively low pH (6.5), and N availabilities would also be high corresponding to its P availability. We tested the hypothesis that soil N and P availabilities, and N and P concentrations in leaves and leaf litter, would be higher in the karst site than the non-karst site. We also examined whether this karst ecosystem had lower soil K availability and foliar K concentrations than the non-karst ecosystem to explore the possibility of K deficiency in the karst ecosystem.

Materials and Methods

Study site and tree census

This study was conducted in cool-temperate broad-leaved deciduous forests located on Mount Ibuki in Shiga, Japan (35˚25'04"N, 136˚24'22"E, summit elevation 1,377 m asl). We established plots on both limestone karst and sandstone non-karst sites in south-facing mid-slope areas at approximately 400 m above sea level in 2020. From 2010 to 2019, the mean annual temperature and precipitation at the nearest weather station (located approximately 7 km away in Maibara city) ranged from 13.2℃ to 14.4ºC and 1,418 mm to 2,142 mm, respectively (data from the Japan Meteorological Agency). The limestone of Mt. Ibuki formed during the Permian period and contains the fossils of fusulinids, while the sandstone formed during the Jurassic period (Yamamoto 1985). Sandstone deposited during the Jurassic period is a typical basement rock of the Mino-Tamba belt, which includes Mt. Ibuki (Kimura and Hori 1993). We measured tree diameter at breast height (DBH $\geq$ 5 cm) in the plots (50 m × 30 m on limestone and 30 m × 30 m on sandstone). The limestone plot had a larger basal area than the sandstone plot (38.0 $m^2\ ha^{-1}$ and 31.8 $m^2\ ha^{-1}$, respectively). Both were secondary oak forests dominated by *Q. variabilis*, and the tree species compositions did not remarkably vary between the sites (Fig. S1). These forests were visible in aerial photographs dating back to at least 1946 and were likely used as fuelwood forests until the mid-20th century.

Soil sampling

We collected topsoil (approximately 0-25 cm) and subsoil (approximately 25-50 cm) samples (roughly corresponding to A and B layers, respectively) from the center and four corners of the plots in both July and August 2020. At each location, we selected three sampling points spaced approximately 2 m apart to create a composite soil sample. We analyzed the characteristics of both the topsoil, which supports the majority of fine roots, and the subsoil, which retains the characteristics of the bedrock, to investigate the differences in nutrient dynamics between the two ecosystems established on different bedrocks.

Leaf litter sampling

In December 2020, we collected leaf litter from the O horizon at regular 5-m grid points in a 30 m × 30 m area of the established plots. These litters had fallen during the autumn (October and November) of 2020 and showed no obvious signs of decomposition or soil particle contamination. At each sampling point, we placed a 0.5-m^2 circular frame on the forest floor and collected the leaf litter within it. The quantity of leaf litter per unit ground area was approximately 1.3 times higher in the limestone site, but the litter quantities per unit basal area of trees were similar between sites (data not shown). We took five subsamples of the leaf litter from each plot and dried them at 65 ºC for three days.

Leaf sampling

We selected four tree species, three deciduous (*Carpinus tschonoskii*, *Cornus macrophylla*, and *Q. variabilis*) and one evergreen (*N. sericea*), that were common to both study sites. The individuals from which we harvested leaf samples were larger than 5 cm in DBH, with mean DBHs of 19.3 and 18.4 cm in limestone and sandstone plots, respectively. In August 2020, we collected live leaf samples from the lower canopy layer of four individuals per species at each site. The mass-basis concentrations of foliar nutrients that we focused on in this study are generally similar between sun-exposed and shade leaves, while leaf mass per area and area-based nutrient contents are higher in sun-exposed leaves (Poorter et al. 2006, Rozendaar et al. 2006, Markesteijn et al. 2007, Legner et al. 2014). The leaves were dried at 65 ºC for three days in the same manner as the leaf litter.

Chemical analysis of soil

We used the soils collected in July for chemical analysis. Fresh soil was sieved at 2 mm to measure soil pH and other soil characteristics as follows. We measured soil pH in a soil-water mix solution adjusted at specific ratios (1: 2.5 = dry soil mass: ultra-purified water), using a pH meter (HM-30G; TOADKK, Tokyo, Japan). We quantified ammonium-N (NH_4^+) and nitrate-N (NO_3^-) with 2 M KCl extraction (1: 10 = fresh soil mass: the solution) followed by colorimetry with a continuous flow system (AutoAnalyzer, BL-Tech, Tokyo, Japan). The extracted organic carbon (C) and total N with 2 M KCl solution were measured with a TOC/TN analyzer (TOC- L CPH/CPN, Shimadzu, Kyoto, Japan). We calculated the amount of organic N by subtracting the amount of inorganic N from the total N. We measured the concentrations of two different fractions of P in the soil (alkaline-soluble P and acid-soluble P) using the Olsen method (Olsen et al. 1954) and the Truog method (Truog 1930) followed by the molybdate blue colorimetry. In the Olsen method, the fresh soil was mixed with the solution of 0.5 M $NaHCO_3$ (pH = 8.5) at a specific ratio (1: 15 = fresh soil: the solution). In the Truog method, the fresh soil was shaken in the solution of $(NH_4)_2SO_4$ (3 g L^{-1}, pH = 3) (1: 200 = fresh soil: the solution). The absorbance of each extract was measured at 880 nm, with a flow injection analyzer (AQLA-700; Aqualab Co., Ltd., Tokyo, Japan).

Air-dried soil samples were used for the following chemical analyses. We measured C and N concentrations using a CN analyzer (Macro Coder JM1000CN; J-SCIENCE LAB Co. Ltd., Kyoto, Japan). Soil C concentration largely represents the concentration of organic matter, as most of soil C is organic C in wet or humid regions (e.g., Mi et al. 2008). The C to N ratio of soils is generally used as an extent of the progress of the decomposition of organic matter (a low C to N ratio indicates an advanced decomposition), as C is released from organic matter much more rapidly than N during the decomposition (e.g., Madritch and Lindroth 2006). We determined the exchangeable Ca and K concentrations in the soil using atomic absorption spectroscopy (Solaar S2, Thermo Fisher Scientific, Massachusetts, USA) after extraction with 1M ammonium acetate (pH = 7.0). To assess temporal variability during leaf development, we also measured selected characteristics (pH, C, N, and P) of the soils collected in August using the same methods as those used for the July samples.

Chemical analysis of leaves and leaf litters

The dried leaf and leaf litter samples were ground into a fine powder for elemental analyses. We quantified leaf N concentration, using the CN analyzer. We measured leaf P concentration with hot H_2SO_4 digestion followed by the molybdate blue colorimetry. We quantified leaf Ca and K concentrations, using atomic absorption spectroscopy (Solaar S2) after the wet digestion with 60% HNO_3 and 30% H_2O_2 in a microwave digester (Multiwave 3000, Anton Paar GmbH, Graz, Austria).

Statistical analysis

We used a two-way analysis of variance to assess the influence of site, soil layer, and their interaction on soil characteristics. We also performed a two-way analysis of variance to examine the influence of site, species, and their interaction on leaf nutrient concentrations. When the interaction effect was not significant, we conducted the two-way analysis of variance without the interaction term. The differences in leaf litter nutrient concentrations between the sites were analyzed using a *t*-test. Normality and homogeneity of variance were assessed using the Shapiro-Wilk test and Levene's test (car package in R, Fox and Weisberg 2019), respectively. When these assumptions were not met, the variable was log10-transformed. For Truog P, a value of 0.001 was added to each value prior to log10-transformation due to the presence of zero in the dataset. The significance level was set at $p < 0.05$. All the data analyses were performed with R software of version 3.6.3 (R Core Team, 2020).

Results

Soil characteristics

The overall soil characteristics differed between the limestone and sandstone plots. The limestone plot was characterized by higher soil pH, a lower ratio of C to N, higher concentrations of both Olsen- and Truog-P, and exchangeable Ca, and lower exchangeable K concentration than the sandstone plot (Table 1). The availability of inorganic N did not differ between the plots (Table 1).

While most soil characteristics differed by soil layers, the interactions of soil types and layers were not significant except in C: N and Olsen-P (Table 1), suggesting that the differences of most soil characteristics by soil types were consistent across layers. The trend of soil pH, N availability and Olsen-P did not differ between the different collection months (Table S2).

Elemental concentrations of leaf litter and live leaves

The concentrations of Ca, N, and P in leaf litters were higher in the limestone plot than in the sandstone plot, while K concertation showed the opposite trend (Fig. 2). Live-leaf P concentration was higher in the limestone plot than in the sandstone plot, while the opposite trend was observed in leaf K concentration (Table 2, Fig. 3). Ca and N concentrations of the live-leaf were not significantly different between the plots. Leaf N concentrations showed a significant interaction between site and species but no effects of sites and species in the two-way analysis of variance (Table 2), indicating that the difference in leaf N concentrations between sites was not consistent across species.

Discussion

We hypothesized that the soil P availability in this karst forest would be high due to its soil pH of 6.5, which is lower than the deposition of phosphate by Ca ion typically occurs (Lindsay and Moreno 1960) and that soil N and P availabilities and foliar N and P concentrations would be higher in the karst site than the non-karst site. We found that this karst ecosystem was richer in P than the non-karst ecosystem, as we hypothesized (Table 1 and 2, Figs. 2 and 3). Litter N concentration was higher in the karst ecosystem (Fig. 2), consistent with our hypothesis, but availabilities of inorganic N in the soil were similar between the two sites (Table 1). We also tested if this karst ecosystem is poorer in K than the non-karst ecosystem and found that soil K availability and foliar K concentration were lower in the limestone plot (Tables 1 and 2, Figs. 2 and 3).

The concentrations of Olsen- and Truog-P in the soil and foliar P concentrations were higher in the karst ecosystem than in the non-karst ecosystem (Table 1 and 2, Figs. 2 and 3), contrary to the typical karst soils under P deficiency (Niinemets and Kull 2005, Liu et al. 2014). In addition, our data suggest that the karst ecosystem is considerably rich in P. Both Olsen- and Truog-P in this limestone soil (247.9 mg kg^{-1} of Olsen-P and 2288.5 mg kg^{-1} of Truog-P) were even higher than those observed in some fertilized crop fields (e. g. Bai et al. 2013 for Olsen-P, Ando et al. 2021 for Truog-P). Especially, the Truog-P concentration of this karst soil is further higher than the total P concentrations of most other karst soils (Table S1) and most soils in the global data (He et al. 2021). As the concentrations of P extracted under both extractions are high, we believe that the P availability of the studied limestone soil to plants is also considerably high in the field. In addition, the P concentrations of live leaves of the four species in the limestone plot (1.75-3.16 mg g^{-1}, Fig. 3) almost overlap with the higher range of the global data of leaf P concentrations of 923 plant species (median 1.77 mg g^{-1} and third quartile point 2.30 mg g^{-1}) reported by Reich et al. (2004). Furthermore, the litter P concentration in the limestone plot (1.47 mg g^{-1}) was equal to the upper 15 percentile of the 3087 litter P concentrations in global data reported by Xie et al. (2022). This large amount of P in the karst ecosystem might be derived from the calcium phosphate in the deeper soil, given a higher Truog-P concentration in the subsoil than the topsoil in the limestone plot (Table 1). We suspect that this limestone soil releases much P under its soil pH, in which the solubility of calcium phosphate is high (Lindsay and Moreno 1960). In this study, we found a negative correlation between Olsen-P concentration and pH in the subsoil of limestone soil (Fig. S2). For Olsen-P concentration in topsoil, we found a significant quadratic relationship with soil pH ($p < 0.001$, Fig S2), indicating that Olsen-P concentration in topsoil becomes the highest at certain soil pH (6.4 in the vertex of the quadratic curve). On the other hand, we did not find a significant relationship between Truog-P concentration and soil pH regardless of soil layers (Fig. S2). The within-site variation of the soil pH of a karst site (5.8 – 7.3 in topsoil and 5.0 – 7.9 in subsoil, Fig. S2) suggests that the degree of decalcification is considerably heterogeneous even within the site. Such heterogeneity could have been caused by the scattering stones of limestone *in situ*. P-richer karst ecosystems than the neighboring non-karst ecosystems have also been reported by Fu et al. (2019), Rossatto et al. (2015), and Zhang et al. (2019), although their soil P availabilities are lower than in this karst forest. However, whether the karst ecosystem is richer in P than the neighboring non-karst does not necessarily relate to soil pH (Table S3). This might be partly because the solubility of P is determined not only by soil pH but also by the concentrations of cations and anions in the soil solution (Hinsinger 2001). For example, P solubility at the same pH of solutions may increase several folds with the increasing concentration of citrate ion (Gerke et al. 2000) and approximately 1.5-2.0 times with the decreasing concentration of Ca ion (Lindsay and Moreno 1960). Nevertheless, if other conditions are similar, soil pH should be a primary determinant of the solubility of the P in the soil. Indeed, in calcareous dunes in the Netherlands, a higher soil P availability is observed in more decalcified soils with lower pH even though the concentration of inorganic P (P bound to metal ions) is higher in less decalcified soil with higher pH (Kooijman et al. 2021).

This karst ecosystem seems richer in not only P but also N than the non-karst ecosystem, as the N concentration of leaf litter was higher in the limestone site (Fig 2). Though our results showed that inorganic N availabilities did not differ between the two sites (Table 1), it is possible that the mineralization rate of N is higher in the limestone site, given that the soil C:N ratio, which reflects the progress of litter decomposition (e.g., Madritch and Lindroth 2006), was lower in the limestone site than in the sandstone site (Table 1). The lower soil C:N ratio in the karst site is in line with the result of a recent study conducted at the same sites by Nakamura et al. (2023) that the abundance of bacteria in the soil is higher in the karst site than in the non-karst site. Moreover, as the rates of N mineralization and nitrification increase in the presence of high P availability (DeForest and Otuya 2021, Sasaki et al. 2022), it is also possible that these processes are more active in the limestone site. Such inorganic N could be absorbed by trees more rapidly in the limestone site, and it might result in a similar amount of inorganic N extracted in this study.

The karst ecosystem was richer in Ca but poorer in K than the non-karst ecosystem, reflecting the elemental composition of the limestone. As this limestone plot showed similar or even lower foliar K concentration (4.7-7.7 mg g^{-1} in live leaves, Fig. 3 b, and 2.2 mg g^{-1} in leaf litter, Fig. 2 b) than the karst ecosystems under K deficiency (Liu et al. 2014, Zhu et al. 2021) and/or co-limitation of N, P, and K (Liu et al. 2018), we suspect that the karst ecosystem on Mt. Ibuki might also be under K deficiency. The generality of K-deficient conditions in karst ecosystems should be further tested in the future. This karst site also showed high soil Ca availability and litter Ca concentration (Fig.2, Table 1) like many other karst ecosystems (e.g., Wei et al. 2018, Zhu et al. 2021). Though the Ca concentrations of live leaves did not differ significantly between the two sites (Table 2), the leaf Ca concentration of each species was apparently higher in the limestone site except for *Cornus macrophylla* (Fig. 3). A high Ca availability in the limestone soil and a high Ca concentration in leaf litter in the limestone plot on Mt. Ibuki indicates that this limestone soil is still rich in Ca, unlike the decalcified one observed by Ueno (2013), located approximately 15 km away from Mt. Ibuki.

Implications to P availability in karst soils at low pH

Our findings extend our understanding of karst ecosystems by demonstrating that limestone soil can release significant amounts of P for plants at relatively low pH (6.5). In addition, karst soils in Japanese temperate zones often have lower pH than typical karst soils (pH < 7.0, Hayakawa 2007, Ueno 2013) probably due to high precipitation in the region, which accelerates the loss of mineral elements from the soil (Vitousek and Chadwick 2013). Thus, there could be other karst ecosystems with such high P availabilities in this region, though most karst soils with relatively low soil pH might be already decalcified like the one observed by Ueno (2013). Calcium phosphate occupies most of the soil P when soil is newly established (Walker and Syes 1976) and its amount decreases with time in both calcareous (Ryan and Zghard 1980) and non-calcareous soils (Syes and Walker 1969). Calcium phosphate in this limestone soil seems to release a large amount of P in the middle of the biogeochemical processes that decrease soil pH, such as the loss of mineral elements or the exudation of organic acid from roots and decomposed litter. Furthermore, the high concentration of Olsen-P in this limestone soil suggests that much P was bound to also Al and/or Fe. Because our data lacks plot replicates, verifications across multiple sites are necessary to understand P dynamics in karst soils during weathering. The initial P concentration of karst soils (i.e., the P concentration of the bedrock) should differ by the types of limestone (e.g., from which organisms the limestone is derived). It would be valuable to examine how soil P availability and other characteristics of karst ecosystems change with biogeochemical processes by comparing the chronosequence of karst soils derived from the same bedrock.

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Conflicts of interest

We declare no conflict of interest.

Author contribution

HK, MF, and RN conceived the ideas and designed the methodology; HK, MF, YF, RF, NO, and RN conducted a field survey; HK, MF, YF, RF, TY, CT, TS, and RN performed chemical analyses; MF and RN analyzed the data; HK and RN led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Supplementary Information

Supplementary Information will be available online.

Tables

Table 1. Chemical characteristics of topsoil and subsoil. The effects of sites (limestone vs. sandstone) and layer (topsoil vs. subsoil) were tested by the analysis of variance (ANOVA).

Table 2. Analysis of variance (ANOVA) results for the effects of the site (limestone vs. sandstone) and species on leaf nutrient concentrations.

Table 1. Chemical characteristics of topsoil and subsoil. The effects of sites (limestone vs. sandstone) and layers (topsoil vs. subsoil) were tested with ANOVA.

	Limestone		Sandstone		ANOVA results		
	topsoil	subsoil	topsoil	subsoil	site	soil layer	site × soil layer
Soil pH	6.5 (± 0.5)	6.6 (± 1.0)	5.6 (± 0.4)	5.5 (± 0.4)	**	ns	ns
%C	9.1 (± 1.8)	2.9 (± 1.1)	9.9 (± 1.8)	4.2 (± 1.6)	ns	***	ns
%N	0.7 (± 0.2)	0.3 (± 0.1)	0.6 (± 0.1)	0.3 (± 0.1)	ns	***	ns
C:N	12.8 (± 0.9)	9.5 (± 0.3)	15.3 (± 0.7)	14.0 (± 1.2)	***	***	*
NO_3^- (mg N kg^{-1})	11.2 (± 5.4)	1.4 (± 1.7)	16.0 (± 7.4)	5.1 (± 5.1)	†	***	ns
NH_4^+ (mg N kg^{-1})	33.9 (± 7.7)	7.7 (± 3.7)	34.9 (± 10.4)	9.8 (± 4.2)	ns	***	ns
Inorganic N (mg N kg^{-1})	45.2 (± 11.4)	9.1 (± 4.5)	50.9 (± 13.4)	15.0 (± 8.9)	ns	***	ns
Olsen-P (mg kg^{-1})	247.9 (± 38.5)	104.9 (± 48.0)	60.1 (± 22.5)	27.4 (± 6.6)	***	***	**
Truog-P (mg kg^{-1})	2288.5 (± 892.3)	3165.6 (± 1662.5)	19.6 (± 22.7)	7.9 (± 11.1)	***	ns	ns
Ca (g kg^{-1})	6.3 (± 2.2)	3.9 (± 2.5)	3.3 (± 1.2)	1.2 (± 0.7)	**	*	ns
K (g kg^{-1})	0.51 (± 0.1)	0.21 (± 0.1)	0.68 (± 0.1)	0.41 (± 0.1)	***	***	ns
Water extractable organic C (mg kg^{-1})	411.8 (± 204.4)	219.4 (± 57.4)	547.6 (± 221.0)	248.4 (± 88.1)	ns	**	ns
Water extractable organic N (mg kg^{-1})	82.5 (± 21.0)	46.7 (± 10.1)	101.2 (± 17.5)	52.3 (± 17.6)	ns	***	ns

Note: †$P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Inorganic N is a total of NO_3^- and NH_4^+. Soil samples were collected in July. Soil depth (cm): 0-27 cm for topsoil and 27-50 cm for subsoil at the limestone site, 0-25 cm for topsoil and 25-40 cm for subsoil at the sandstone site. Truog-P and water-extractable organic C were log_{10}-transformed prior to the test.

Table 2. Analysis of variance (ANOVA) results for the effects of sites (limestone vs. sandstone) and species on leaf nutrient concentrations.

	site			species			site × species		
	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Ca (mg/g)	1	1.3	0.25	3	16.5	**< 0.001**		ns	
K (mg/g)	1	19.3	**< 0.001**	3	3.3	**< 0.05**		ns	
N (mg/g)	1	1.2	0.28	3	2.6	0.08	3	3.7	**< 0.05**
P (mg/g)	1	178.5	**< 0.001**	3	11.3	**< 0.01**	3	11.2	**< 0.001**

Note: significant terms ($P < 0.05$) are shown in bold. We $\log_{10}$-transformed K and P prior to the test.

Figure legends

Figure 1. A conceptual diagram of the hypothesis of this study.

Figure 2. Leaf litter concentration of (a) Ca, (b) K, (c) N, and (d) P in the plots of limestone (white) and sandstone (grey). The P value from the *t*-test is provided in each panel: *P < 0.05, **P < 0.01, ***P < 0.00. Mean ± s.d. (n = 5). Leaf-litter N was log10-transformed to improve normality prior to the test.

Figure 3. Leaf concentration of (a) Ca, (b) K, (c) N, and (d) P in the plots of limestone (white) and sandstone (grey). Three deciduous species (*Carpinus tschonoskii, Cornus brachypoda,* and *Quercus variabilis*) and one evergreen species (*Neolitsea sericea*) were selected. Mean ± s.d. (n = 4).

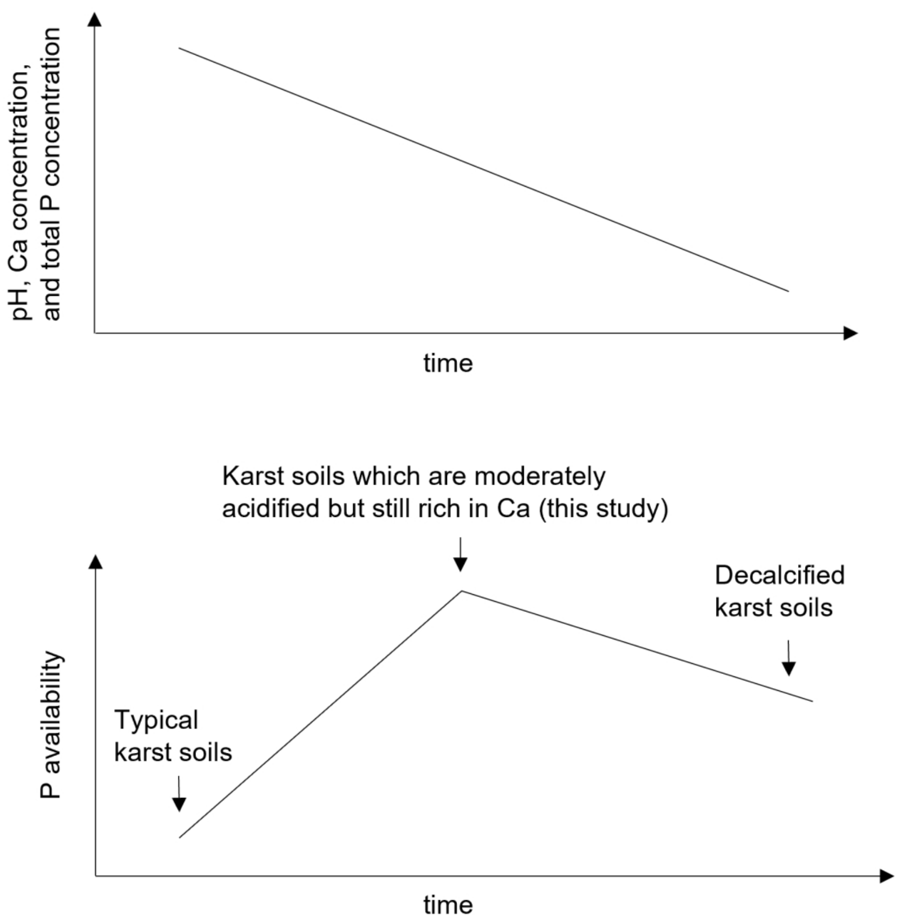

Figure 1. A conceptual diagram of the hypothesis of this study.

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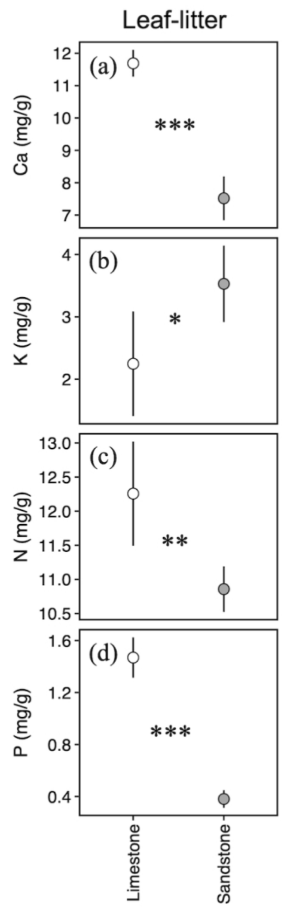

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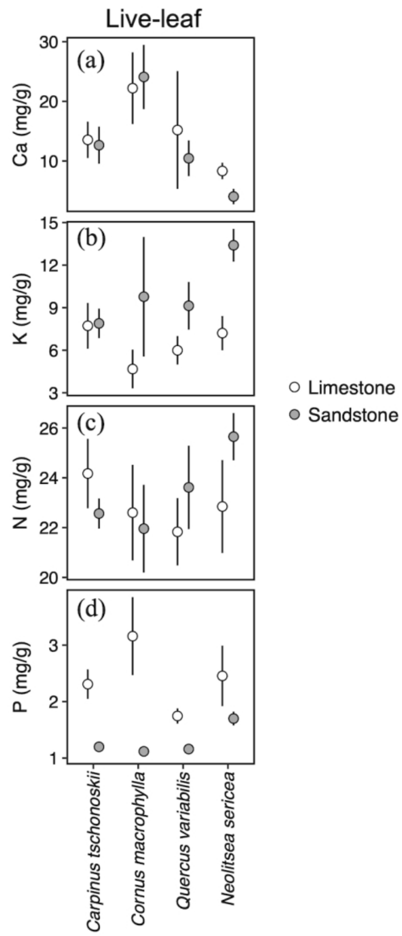

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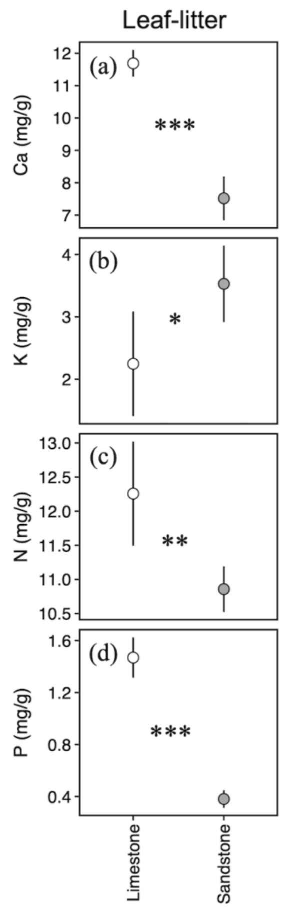

GTOC: We explored the soil nutrient availabilities and foliar nutrient concentrations in a karst forest with relatively low soil pH on Mt. Ibuki, in Japan. We found that the karst ecosystem is considerably rich in P, contrary to other typical karst ecosystems with high soil pH and low P availabilities.

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Supplementary Information

Variations in soil nutrient availabilities and foliar nutrient concentrations of trees between temperate monsoon karst and non-karst forest ecosystems on Mount Ibuki in Japan

Ecological Research

Hirofumi Kajino, Misaki Fukui, Yutaro Fujimoto, Rei Fujii, Tomohiro Yokobe, Chikae Tatsumi, Tetsuto Sugai, Naoki Okada, Ryosuke Nakamura

Content

Table S1 The summary of previous studies which reported the mass-basis concentrations of total P and/or available P in the topsoil of karst soils. For available and/or soluble P, the solutions used for the extraction are also shown. Note that Zhu et al. (2022) conducted a meta-analysis of karst soils in southwest China that includes the data of soil total P by Zhang et al. (2015).

References	Region	Soil pH	Total P concentration (mg kg^{-1})	Available and/or soluble P concentration (mg kg^{-1})	Extraction solution of available and/or soluble P	Relevant figures or tables in articles
Wilson et al. (1995)	UK	8.1	1320	18.5	not described	Table 2
Hofmeister et al. (2001)	Czech and Slovenia	4.6 -7.5 in Czech, 4.8 - 6.4 in Slovenia	NA	3 - 162 in Czech, 7 - 15 in Slovenia	concentrated HNO_3, NH_4NO_3, concentrated CH_3COOH, NH_4F, and EDTA	Table 2, and 3
Köhler et al. (2001)	Switzerland	7.2	567 - 558	NA	NA	Table 1

Niinemets and Kull (2006)	Estonia	6.9	NA	14.9	lactic (0.1 M) and acetic (0.3 M) acids, and ammonium acetate (0.1 M; pH 3.75)	Table 1
Zhang (2006), Fu et al. (2019)	China	7.4	1230	20.9	0.03 mol/LNH4F – 0.025mol/L HCl	Table 4 in Zhang (2006), and Table S1 in Fu et al. (2019)
Storm and Süss (2008)	Germany	7.4	NA	14.9	0.05 mol/L calcium acetate, 0.05 mol / L calcium lactate, and 0.3 mol/L acetic acid	Table 1
Du et al. (2011)	China	6.9 - 7.8	190 - 280	1.7 - 4.0	0.5 M sodium bicarbonate	Table 1
Ueno (2013)	Japan	6.4	743 - 1530	NA	NA	Table 12 (originally described in P_2O_5 basis)
Liu et al. (2014)	China	NA	680 - 1070	NA	NA	Table S1
Hao et al. (2015)	China	7.6	1620	NA	NA	Table 1

Zhang et al. (2015)	China	6.8 - 7.3	770 - 1660	3.7 - 12.4	0.5 M sodium carbonate	Table 2
Chen et al. (2017)	China	6.6	2000	6.3	0.5 M sodium carbonate	Table 2
Liu et al. (2018)	China	7.7	900	6.3	sodium bicarbonate	Table 1
Zhu et al. (2021)	China	6.6	NA	0.67	HCl-NH4F	Table 1
Zhu et al. (2022)	China	4.1 – 9.5 (mean 6.7)	150 – 4050 (mean 930)	NA	NA	Table 1

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Table S2 Chemical characteristics of topsoil and subsoil at a different collection month (August). The effects of the sites (limestone vs. sandstone) and layers (topsoil vs. subsoil) were tested with ANOVA.

	Limestone		Sandstone		ANOVA results		
	topsoil	subsoil	topsoil	subsoil	site	soil layer	site × soil layer
Soil pH	6.5 (± 0.6)	6.5 (± 1.0)	5.6 (± 0.5)	5.2 (± 0.6)	**	ns	ns
NO_3^- (mg N kg^{-1})	19.2 (± 10.2)	4.6 (± 2.6)	19.5 (± 10.3)	12.8 (± 11.1)	ns	*	ns
NH_4^+ (mg N kg^{-1})	36.4 (± 10.8)	11.6 (± 2.1)	31.0 (± 7.6)	12.8 (± 3.1)	ns	***	ns
Inorganic N (mg N kg^{-1})	55.5 (± 5.7)	16.3 (± 2.7)	50.6 (± 15.8)	25.6 ± (13.6)	ns	***	ns
Olsen-P (mg kg^{-1})	202.6 (± 40.6)	117.8 (± 45.9)	56.9 (± 17.5)	36.1 (± 4.1)	***	**	*
Water extractable organic C (mg kg^{-1})	320.6 (± 55.7)	348.8 (± 151.0)	409.4 (± 123.3)	361.0 (± 133.6)	ns	ns	ns
Water extractable organic N (mg kg^{-1})	75.3 (± 21.0)	70.6 (± 23.0)	93.2 (± 23.3)	73.8 (± 20.9)	ns	ns	ns

Note: †P < 0.1, *P < 0.05, **P < 0.01, ***P < 0.001. Soil samples were collected in August. Inorganic N is a total of NO_3^- and NH_4^+. Soil depth (cm): 0-27 cm for topsoil and 27-50 cm for subsoil at the limestone site, 0-25 cm for topsoil and 25-40 cm for subsoil at the sandstone site.

Table S3 The summary of previous studies which compared soil P availability and/or P concentration in the plant bodies between karst and the neighboring non-karst ecosystems.

References	Region	The pH of the karst soil	Soil available P	P concentration of plant bodies (i.e., leaf, stem, or root)	Soil types of the non-karst soil	Relevant figures or tables in the article
Ueno (2013)	Japan	6.4	NA	karst ≒ non-karst	sandstone soil	Table 11
Hao et al. (2015)	China	7.6	NA	karst ≒ non-karst	sedimentary soil, shale soil, and granite soil	Table 3
Rosatto et al. (2015)	Brazil	6.64	NA	karst > non-karst	Many types of soils in the Atlantic and Amazon rainforests	Table 5
Chen et al. (2018)	China	6.6	karst < non-karst	NA	Clasolite soil	Table 2
Fu et al. (2019)	China	7.4	karst > non-karst	karst > non-karst	not described	Table S1 for the soil, and Tables 1, 2, and Fig. 2a for the leaf

Zhang et al. (2019)	China	7.5 – 8.0	NA	karst > non-karst	yellow soil	Fig. 2
Tang et al. (2021)	China	6.3-7.8	NA	karst ≒ non-karst	granite soil	Table 2 and Fig. 4
Zhu et al. (2021)	China	6.6	karst < non-karst	karst < non-karst	not described	Table 1 for the soil and Table 4 for the leaf litter (described as P use efficiency, the inverse of litter P concentration)

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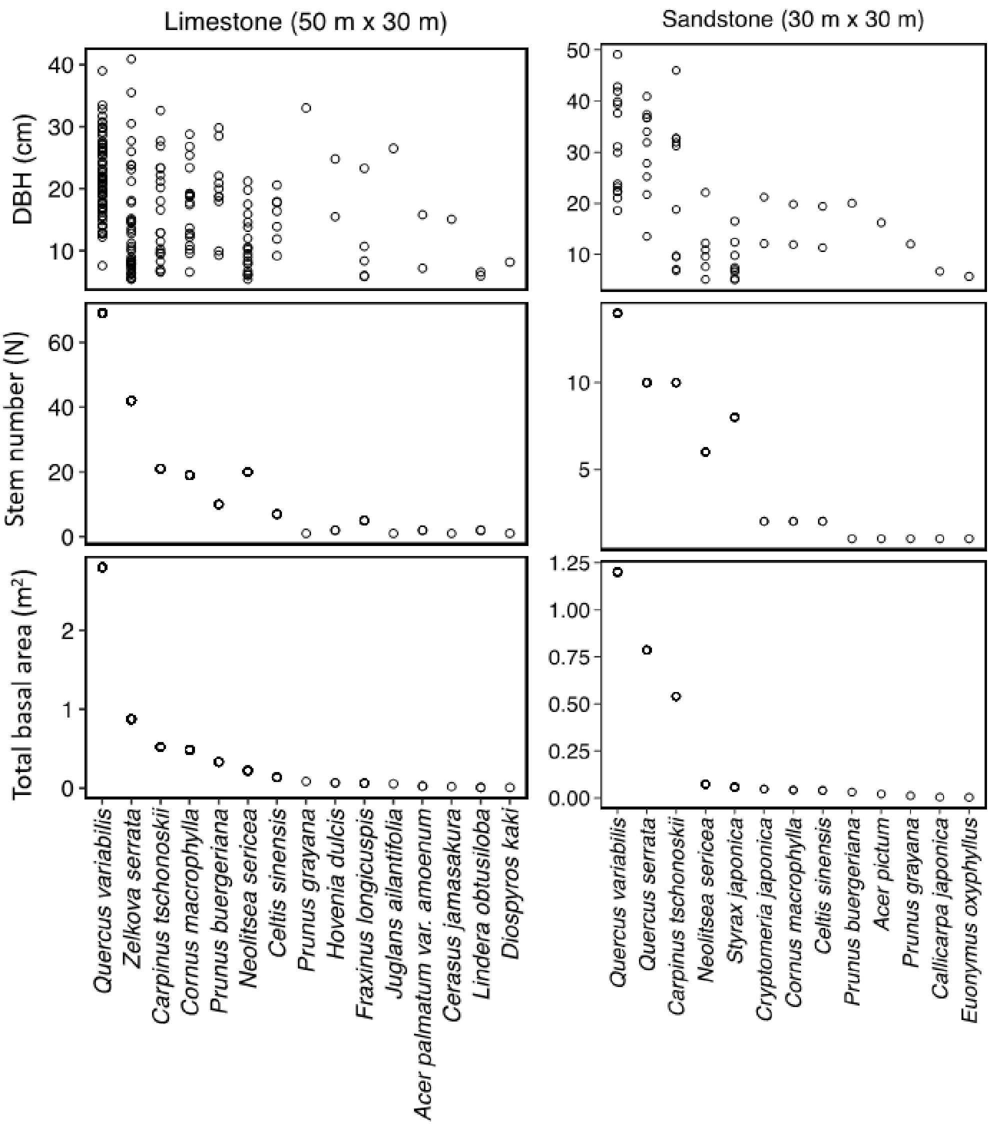

Figure S1. Results of tree census. The DBH, number of stems, and total basal area are shown. The 203 and 59 stems were recorded in the limestone and sandstone plots, respectively.

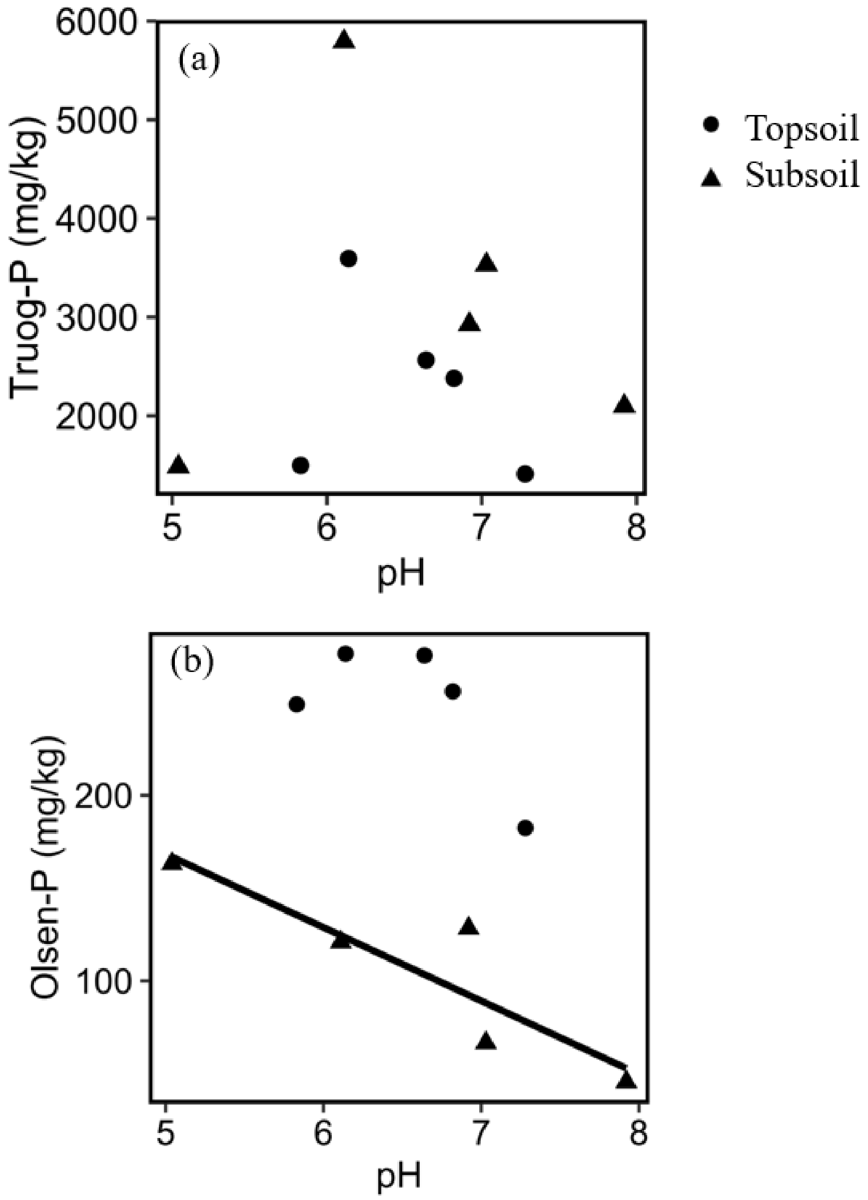

Figure S2. Relationships of Truog-P (a) and Olsen-P (b) with soil pH of the topsoil (circle) and subsoil (triangle) of the karst forest. The regression line was shown only for the Olsen-P of the subsoil, which showed a significant correlation with the soil pH.