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Research Article

Variation of Free Amino Acids in Soil Water of Tropical Rainforests on Mount Kinabalu, Borneo

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ABSTRACT

Despite free amino acids in soil water being an important precursor of inorganic nitrogen (N), little is known about their concentration and spatial variability in tropical rain forests. We investigated the concentration of free amino acids and NH_4^+ in water extracts of soils from nine tropical rain forests, which were widely different in altitude (700 to 3100 m) and soil type on Mount Kinabalu, Borneo. Earlier studies indicated that net N mineralization rate was distinctly high in the two 700-m lowland forests and the 1800-m montane forest on Quaternary colluvial deposits, and consistently low in the other upland forests among the nine forests. Soils were collected from an organic horizon (0–5 cm) and a mineral horizon (5–15 cm) in each of the nine sites. Free amino acids in soil were analyzed by high performance liquid chromatography. Mean total N as amino acids (TNAA) ranged from 0.25 to 7.93 μg/g-dry soil in organic soils and generally decreased with increasing altitude except for the 1800-m montane forest on Quaternary colluvial deposits where mean TNAA and decay constant were outstandingly high. Mean quotient of TNAA to NH_4^+-N was higher (>0.6) in the two lowland forests and in the Quaternary colluvial montane forest, while it was lower (<0.3) in the other upland forests. Our results suggest that slower decomposition of organic matter may retard the supply of amino acids for heterotrophic microbes, which becomes a bottleneck of downstream N mineralization in the upland forests.

Keywords: Altitude; free amino acids; nutrient cycling; N mineralization, tropical rain forest soils.

INTRODUCTION

Availability of inorganic nitrogen (N) for plants is lower in high-latitude terrestrial ecosystems such as Arctic tundra and taiga than in the tropics because net N mineralization in soils is suppressed under a cold climate (Chapin, 1983). It is believed that the productivity and structure of plant communities are limited by N availability in Arctic ecosystems (Chapin, 1983; Kielland, 1995; Schimel et al., 1996). However, N limitation also occurs in several tropical ecosystems where net N mineralization is retarded due to local biophysical conditions such as lowland heath forests and upland forests. It is believed that soil N availability is much lower than tree demand in the heath forest due to slow N mineralization being suppressed by its extremely acidic soil condition (Proctor et al., 1983; Brearley et al., 2011), and in tropical upland forests due to slow N mineralization being suppressed by cold climate (Kitayama et al., 1998).

N dynamics of tropical upland forests in the Asian tropics were intensively studied along an altitudinal gradient of Mount Kinabalu (4095 m), the highest tropical mountain in Southeast Asia (Kitayama et al., 1998; Kitayama & Aiba 2002; Hall et al., 2004; Kitayama et al., 2004). They consistently showed that in-situ net soil N mineralization rate (net production rate of $NH_4^+ + NO_3^-$) decreased with increasing altitude and became nearly zero or negative in upland forests (lower montane, upper montane and subalpine forests), suggesting that N supply was smaller than tree demand for N in these forests (Kitayama et al., 1998). Noteworthy was the influence of geological substrate on net soil N mineralization. Soils derived from sedimentary rocks demonstrated a greater net N mineralization rate than soils derived from ultrabasic rocks at comparable altitude (Kitayama et al., 1998). Moreover, soils derived from young colluvial deposits of Quaternary origin (approximately 30000 years ago) demonstrated an outstandingly high net N mineralization rate (Hall et al., 2004; Kitayama et al., 2004). It was suggested that phosphorus deficiency limited N transformation in ultrabasic soils while phosphorus abundance enhanced N transformation in Quaternary soils. Experimental manipulation with doses of N fertilization indicated that microbial processes were not N-limited in the lowland forests and the montane forest on Quaternary colluvial deposits, while severely N-limited in the other upland forests (Hall et al., 2004).

N mineralization proceeds with the ammonification of amino acids into NH_4^+, followed by nitrification to NO_3^-, which occurs as soil microbial catabolism and anabolism. Regulation of net N mineralization rate was commonly investigated by focusing on these two microbial processes. However, it has been pointed out that proteolysis of high- and low-molecular organic N compounds in soil water (or on soil matrix) also influences net N mineralization rate because the supply of amino acids can regulate the mineralization rate in the downstream pathway (Jones & Kielland, 2002, 2012). It is possible that the reduced availability of inorganic N ($NH_4^+ + NO_3^-$) is due to the slower supply rate of amino acids combined with slower proteolysis of organic N but not due to slower ammonification and nitrification in tropical forests of Kinabalu. Past studies demonstrated that the pool of free amino acids in soil water was very small, ranging from 1 to 50 mM equivalent on a dry soil weight basis, but it turns over very quickly, suggesting that amino acids are an important component in N dynamics (Kielland, 1995; Jones & Kielland, 2002; Jones et al., 2009). Furthermore, Jones & Kielland (2002, 2012) suggested that the transformation of proteins to amino acids, but not amino acids to NH_4^+, is limiting N availability in a taiga forest, indicating that the proteolysis of organic N is still a bottleneck for net N mineralization in this ecosystem despite their fast turnover.

Studies on the pool of free amino acids in soil water and leachates are limited to high-latitude ecosystems only and have not systematically been conducted in the tropics to our knowledge except for Jones et al. (2009). The concentration of free amino acids in soil water (leachates) and their roles in N budget were investigated in Arctic tundra soils in Alaska (Kielland 1995; Weintraub & Schimel, 2005), black-spruce taiga forests in Alaska (Jones & Kielland 2002, 2012), mixed conifer forests and pygmy conifer forests in the Ecological Staircase located in northern California (Yu et al., 2002), temperate Taibai Mountain in China (Cao et al., 2016), along a temperate-forest fertility gradient in Michigan (Rothstein, 2009), and along a global latitudinal gradient (Jones et al., 2009). The pool size of free amino acids and other dissolved organic N in soil water was investigated particularly well in tundra and taiga because a number of tundra and boreal plant species were proven to directly absorb low-molecular weight organic N dissolved in soil water such as free amino acids (Chapin et al., 1993; Kielland, 1994; Jones et al., 2005; Kielland et al., 2006; Hill et al., 2011). There was no evidence that tropical trees on Mount Kinabalu directly absorb dissolved amino acids in soil water. Ushio et al. (2017) fertilized dominant trees with an amino acid solution labelled with carbon (C) and N stable isotopes in an N-limited montane forest on Kinabalu and found that fertilized plants were enriched with N stable isotope only. This study suggested that applied amino acids were mineralized before its N was acquired by plants, implying that the production of amino acids primarily regulate downstream mineralization processes. Therefore, examining amino acids provides a useful means to assess potential bottlenecks in N cycling. In our paper, we address the importance of free amino acids in soil water as the substrate for the downstream ammonification but not as the direct source of N to tropical trees.

We here report the composition and concentration of free amino acids in water extracts of soils from nine tropical forests that differ in altitude and geological substrate. We ask the following research questions in this study. 1) How does the composition of free amino acids in soil water spatially vary in relation to altitude and geological substrate? 2) How does the quotient of total N as amino acids to NH_4^+ spatially vary and relate to net soil N mineralization? The quotient of total N as amino acids to NH_4^+ represents a ratio of substrate (i.e. amino acids) to product (i.e. NH_4^+) in N mineralization and indicates how or if the production of amino acids regulates downstream processes. It must be noted this model assumes a constant nitrification potential across sites because greater nitrification potential (i.e. consumption rates of saturated NH_4^+) decreases the concentration of NH_4^+ and hence overestimate the quotient of total N as amino acids to NH_4^+. With this limitation, we use the quotient as first approximation only. Addressing the second question can help in elucidating the control of N cycling in tropical ecosystems.

METHODOLOGY

Study sites

Mount Kinabalu, Sabah, Malaysia, is the highest mountain (4095 m) in Southeast Asia between the Himalayas and New Guinea. Pristine forests occur from 300 to 3700 m with the latter altitude demarcating a forest limit. Vegetation is divided to lowland tropical rain forest below 1200 m, lower montane tropical rain forest (1200–2350 m), upper montane tropical rain forest (2350–3100 m) and tropical subalpine forest (3100–3700 m) (Kitayama, 1992). Climate is humid tropical with weak influences of the annual shift of ITCZ (Inter Tropical Convergence Zone) (Kitayama et al., 2020). Mean annual air temperature is 27.5^{o}C at 0 m and decreases linearly with increasing altitude with a lapse rate of 0.55^{o}C per 100 m. Mean annual rainfall is relatively invariable along the altitudinal gradient, ranging from 2300 to 2900 mm/yr.

Geology is complex on Mount Kinabalu (Aiba & Kitayama, 1999; Kitayama & Aiba, 2002). Tertiary sedimentary rocks occur mostly below 2700 m on the south face, while granitic rocks dominate the summit area above 3100 m. Ultrabasic rocks which are predominantly serpentinites penetrate between 2700 and 3100 m, and also occur in a mosaic manner at lower altitudes. In addition, colluvial deposits of Quaternary origin (approximately 30000 years ago) cover a large area between 1700 and 2000 m on the south face; colluvial deposits consist of sedimentary rocks with a mixture of some ultrabasic rocks. Soils derived from these rocks are different in nutrient availability. Soils derived from ultrabasic rocks contain a lower amount of phosphorus (P) compared with soils derived from sedimentary rocks at the same altitude and ultrabasic forests commonly show oligotrophic physiognomy. Soils derived from Quaternary colluvial deposits are much richer in soil P, N and cations (Kitayama et al., 2004).

A total of nine study sites were established by making use of the steep altitudinal gradient and complex geology by earlier workers. We used these sites in our study (Table 1). Four principal altitudes were selected along the altitudinal gradient at 700, 1700, 2700 and 3100 m. A pair of two forests were selected at each altitude, one on Tertiary sedimentary soils and the other on ultrabasic soils. The "sedimentary" forest at 3100 is underlain by granitic rocks. Additionally, one forest on Quaternary colluvial deposits was selected at 1800 m (hereafter Quaternary montane forest). The exact altitude of each forest is indicated in Table 1.

Table 1: Description of the nine forests that were analyzed for free amino acids in soils on Mount Kinabalu.

Site name	Common altitude (m)	Exact altitude (m)	ANPP ($g/m^2/yr$)	Mean net N mineralization (µg/g/10d)	Soil pH (water)	Soil C/N ratio	Decay constant	Description
Sedimentary								
PO	700	650	1913	19.9	4.1	13.8	1.69	Lowland forest
PHQ	1700	1560	1110	-0.01	4.0	13.7	1.18	Montane forest
RTM	2700	2590	780	-1.3	3.4	19.2	1	Montane forest
PAKA	3100	3080	816	5.5	4.9	14.3	0.87	Subalpine forest (granite)
Ultrabasic								
NAL	700	700	1715	8.5	4.5	11.4	1.67	Lowland forest
BB	1700	1860	813	1.6	5.4	12.1	0.71	Montane forest
CC	2700	2700	725	0.4	5.1	9.9	0.8	Montane forest
HP	3100	3050	199	-2.2	5.3	13.3	0.44	Subalpine forest
Quaternary								
BU	1700	1860	1230	19.0	4.2	13.0	1.53	Montane forest

Net N mineralization rate was continually investigated in the nine sites by earlier workers (Kitayama et al., 1998; Aiba & Kitayama, 1999; Kitayama & Aiba, 2002; Kitayama et al., 2004; Hall et al., 2004). Results consistently demonstrated that net mineralization rate was

greater in the two lowland forests at 700 m and in the Quaternary montane forest than in the other six sites. Mean net N mineralization rate was 19.9 (μg/g/10d) in the sedimentary lowland forest, 8.5 (μg/g/10d) in the ultrabasic lowland, and 19.0 (μg/g/10d) in Quaternary montane forest, while it was nearly zero or negative in the other upland forests except for 3100m granitic subalpine forest where net N mineralization was slightly elevated to 5.5 (μg/g/10d). A greater net N mineralization rate was ascribed to high temperature for the two lowland forests, while it was ascribed to greater P availability for the Quaternary montane forest because P up-regulates soil N dynamics (Kitayama et al., 1998; Kitayama et al., 2004; Hall et al., 2004). In the following analysis, we aggregate the three forests with higher net N mineralization and the other forests with lower net N mineralization, and test significant differences between them in total N as free amino acids.

Decomposition rate of litter was estimated using the mass balance method by earlier workers in each site (Kitayama & Aiba, 2002; Kitayama et al., 2004) and we here cite their values (Table 1). Briefly, standing litter was collected using ten small circular plots (44-cm diameter) and determined for oven-dry weight five times in each site. Decay constant (k) was estimated by dividing known rates of litterfall with mean standing litter crop.

Soil sampling

Three 50-m long transects were laid out, each from a random point, in each of the nine sites in February 2000. We collected five 15 cm-deep soil cores beneath litter layer by forcing a core sampler (37 mm diameter) at 10 m intervals along each transect. Each collected core was divided to top organic layer (0–5 cm) and subsoil mineral layer (5–10 cm). Soil samples from each transect were composited by layer, yielding three organic soil composites and three mineral soil composites per site. Collected soils were kept in a cooler box in the field and brought back to the laboratory and transferred to a refrigerator. Fresh composite samples were manually homogenized with roots removed within 5 hours after the soil collection.

A subsample of each composite was extracted for free amino acids with ultra-pure water. The soil:water ratio was 1:10 on a weight basis for organic soils, and 1:4 for mineral soils. Water was used as an extractant because organic solvent or common KCl solution causes microbial lysis and inflates free amino acids (Jones et al., 2002; Kielland, 1995). Soil-water slurries in centrifuge tubes were rigorously shaken for 5 minutes and then centrifuged with 3000 rpm for 5 minutes. Supernatant of each centrifuge tube was filtered with a 0.2-μm pore syringe filter (DISMIC, ADVANTEC, Tokyo). Prior to the filtration, each syringe filter was rinsed with 6-ml ultrapure water and then with 2-ml supernatant. Each filtrate (2 ml in volume) was transferred to a glass vial and used for the following amino acid assay. Another subsample of each soil composite was oven-dried at 105^{o}C for 48h to determine gravimetric water content. Recovery of free amino acids may vary depending on physico-chemical properties of the soil matrix.

Determination of amino acids

We determined a total of 12 amino acids and NH_4^+ in soil extracts. Analyzed amino acids are indicated in Appendix I.

Amino acids in soil filtrates were analyzed using a post-column derivatization method in high performance liquid chromatography (HPLC Amnio Acid Analysis System, Shimadzu, Kyoto). We used a standard mixture which comprised 17 species of known amino acids and NH_4^+ with 2.5 μmol/ml concentration (FUJIFILM Wako Chemicals 018-27881, Tokyo). The standards were diluted 250 times resulting in a final concentration of 10 nmol/ml. Separation of amino

acids were conducted by gradient elution (Shimadzu Amino Acid Analysis Mobile Phase Kit Na-Type; Elution buffer A, sodium citric acid/ethanol, pH 3.2 and Elution buffer B, sodium citric acid/boric acid, pH 10) using a cation exchange column (Shim-pack Amino-Na). Then the separated amino acids were derivatized with o-phthalaldehyde (OPA) reagents (Shimadzu Amino Acid Reaction Liquid OPA Kit; A, liquid sodium hypochlorite/boric acid buffer, and B, liquid OPA). The derivatized amino acids and NH_4^+ were detected with a fluorescence detector (Ex350nm and Ex350nm).

Data analysis

Concentrations of amino acids and NH_4^+ (nmol/L) in extracts were converted to amino-acid-N and NH_4^+-N concentrations on a dry-weight soil basis (µg-N/g). We developed a data matrix of 12 amino acids + NH_4^+ and 27 composite samples each for organic soils and mineral soils. Here, zero values were assigned to non-detected amino acids. Subsequently, principal component analysis (PCA) was performed using absolute N concentrations of amino acids in the composite samples. For ordination, only samples from the organic soil horizon (0–5 cm depth) comprising 27 soil composites from the nine sites, were used. Sites were plotted according to their scores along the first two principal components, and the loadings of amino acids were visualized on the respective principal components.

All amino-acid-N concentrations were summed to derive the total N as amino acids (TNAA) in each composite sample. Mean TNAA was calculated for organic and mineral soils separately in each site. Then, the quotient of TNAA to NH_4^+-N was calculated for each composite sample.

To test significant differences in TNAA to NH_4^+-N quotients and mean TNAA concentrations in the soil organic and mineral horizons between the two aggregated forest groups, i.e. high net N mineralization (NAL, PO, BU) versus low net N mineralization (BB, CC, HP, PHQ, RTM and PAKA), a one-way ANOVA was conducted. All data were log-transformed prior to analysis. Statistical tests were conducted using R ver. 4.2.0 (R Core Team, 2024).

RESULTS

Variation of amino acids concentration

Concentrations of extractable N as free amino acids (µg/g) and extractable NH_4^+-N, and quotient of TNAA to NH_4^+-N in soil water extracts are shown in Appendix II. The most frequently found free amino acid was glutamic acid (GLU), followed by aspartic acid (ASP), histidine (HIS), alanine (ALA), cysteine (CYS), serine (SER), and phenylalanine (PHE), each of which occurred more than 14 times across 54 composite samples (N=27 organic and N=27 mineral soils).

Mean TNAA ranged from 0.25 to 7.93 (µg/g) for organic soils, and 0.00 to 1.86 (µg/g) for mineral soils (Table 2). The highest value of TNAA was found in the Quaternary montane forest for both organic and mineral soils. The three forests with higher net soil N mineralization rates (the two lowland forests—NAL and PO; and the Quaternary montane forest—BU) had a significantly greater mean TNAA than the other upland forests with lower N mineralization for organic soils (mean TNAA 5.28 versus 0.88; $P<0.001$, one way ANOVA) and mineral soils (mean TNAA 0.74 versus 0.17; $P<0.05$, one way ANOVA). Variations of TNAA could not be explained by soil pH (a linear regression between TNAA and soil pH; $P>0.05$ for both layers), or by soil C/N ratio, which often regulates N mineralization rate (a linear regression between TNAA and C/N ratio; $P>0.05$ for both layers). Quotients of TNAA to NH_4^+-N ranged from

0.10 to 2.29 and were higher in the three forests than the other upland forests for organic soils (mean quotient 1.33 versus 0.21, $P<0.001$, one-way ANOVA) and for mineral soils (0.51 versus 0.05; $P<0.001$, one way ANOVA).

Table 2: Mean and standard errors (SE) of the concentrations of total N as amino acids (TNAA) and NH_4^+-N, and quotients of TNAA to NH_4^+-N per site at each altitude. Upper table (a) indicates organic horizons, and the lower table (b) indicates mineral horizons.

(a) Organic horizon

Site and altitude (m)	TNAA (µg-N/g-dry soil)		NH_4^+-N (µg-N/g-dry soil)		TNAA/ NH_4^+-N	
	Mean	SE	Mean	SE	Mean	SE
Sedimentary						
700 (PO)	2.19	0.47	4.01	0.80	0.60	0.19
1700 (PHQ)	0.84	0.06	3.06	0.66	0.29	0.05
2700 (RTM)	1.91	0.28	6.82	0.52	0.28	0.04
3100 (PAKA)	0.25	0.03	1.14	0.19	0.22	0.01
Ultrabasic						
700 (NAL))	5.73	1.03	2.62	0.32	2.29	0.66
1700 (BB)	1.04	0.11	10.42	0.80	0.10	0.02
2700 (CC)	0.83	0.23	-	-	-	-
3100 (HP)	0.41	0.06	2.75	0.62	0.16	0.03
Quaternary						
1800 (BU)	7.93	1.42	7.32	1.53	1.10	0.14

(b) Mineral horizon

Site and altitude (m)	TNAA (µg-N/g-dry soil)		NH_4^+-N (µg-N/g-dry soil)		TNAA/NH_4^+-N	
	Mean	SE	Mean	SE	Mean	SE
Sedimentary						
700 (PO)	0.14	0.01	0.52	0.08	0.27	0.02
1700 (PHQ)	0.02	0.02	0.65	0.06	0.04	0.04
2700 (RTM)	0.09	0.02	1.61	0.35	0.06	0.00
3100 (PAKA)	0.00	0.00	0.77	0.05	0.00	0.00
Ultrabasic						
700 (NAL)	0.22	0.17	0.60	0.07	0.39	0.33
1700 (BB)	0.69	0.44	7.29	0.76	0.09	0.06
2700 (CC)	0.18	0.04	-	-	-	-
3100 (HP)	0.04	0.03	0.90	0.14	0.04	0.03
Quaternary						
1800 (BU)	1.86	0.08	2.25	0.51	0.88	0.17

Ordination of PCA

Results of PCA analysis for organic soils are indicated in Fig. 1. The first principal component (PC1) accounted for 65.2% of the total variance, while the second principal component (PC2) accounted for 16.5%. Three forests stood out from the other forests in the biplot ordination of PCA; these are the two lowland forests (NAL and PO) and the Quaternary montane forest (BU). The two lowland forests were separated from all other sites along PC1. PC1 was explained by serine (SER), alanine (ALA), glutamic acid (GLU), glycine (GLY), valine (VAL), aspartic acid (ASP), isoleucine (ILE), leucine (LEU) and phenylalanine (PHE); N concentration of these amino acids (not molar abundance) attenuated with increasing PC1. PC1 amino acid scores were negatively correlated with altitude (r=-0.41, $P<0.05$), but the strength of the correlation was only moderate. The Quaternary montane forest (BU) was separated from

all other sites along PC2. PC2 was characterized by greater cysteine (CYS) and histidine (HIS). All upland forests above 1700 m except for the previous three forests were clustered irrespective of soil types and altitudes.

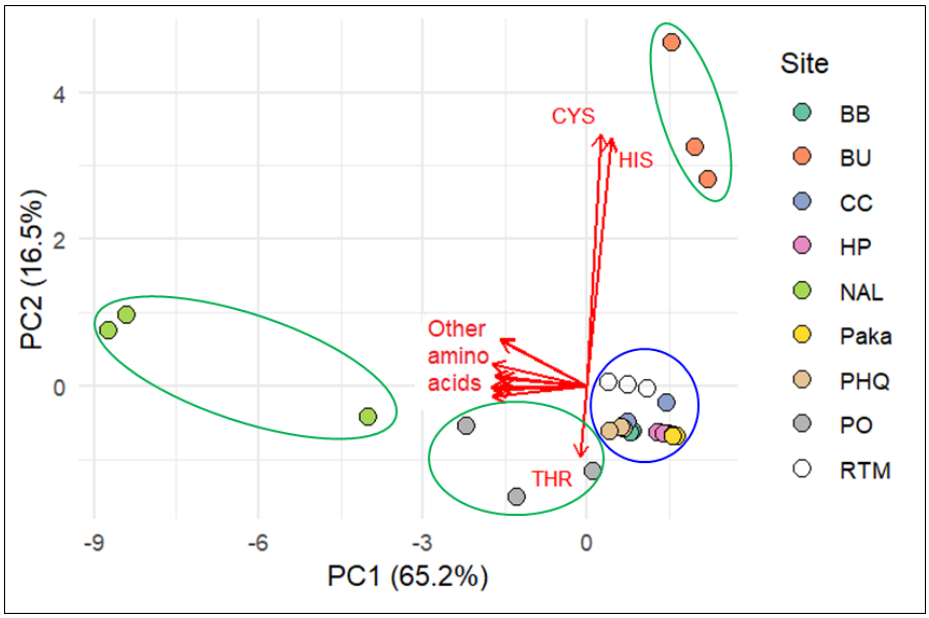

Figure 1: PCA biplot ordination of 27 soil composites from the organic horizon. The other amino acids that are positioned closely together include serine (SER), alanine (ALA), glutamic acid (GLU), glycine (GLY), valine (VAL), aspartic acid (ASP), isoleucine (ILE), leucine (LEU) and phenylalanine (PHE).

DISCUSSION

The three forests stood out from the other upland forests in the biplot ordination of PCA as well as in the mean TNAA concentration; these are the two lowland forests at 700 m (NAL and PO) and the Quaternary montane forest (BU). Concentrations of amino-acid N were greater and thus TNAA was also greater in these three forests than the other upland forests. Conversely all the other upland forests demonstrated lower concentrations of amino-acid N. Among the three forests, the two lowland forests are separated from the other upland forests along PC1, and Quaternary montane forest was separated from the others along PC2. PC1 seems to represent the attenuation of common amino acids except for histidine (HIS) and cysteine (CYS), which implies that the sequence of the two lowland forests and the upland forests along PC1 represents the magnitude of supply and/or consumption (mineralization) of amino acids.

On the other hand, PC2 was characterized by histidine (HIS) and cysteine (CYS). The reason why these two amino acids did not show an attenuating pattern along PC1 and became elevated in Quaternary montane forest needs to be investigated in future. Interestingly, Majuakim & Kitayama (unpublished) found outstandingly high concentrations of these two amino acids in hydrolyzed samples of a hydrophobic fraction in soil water of two montane forests on Kinabalu. Majuakim & Kitayama (2013) suggested that the bulk of hydrophobic fraction consisted of protein (peptide)-polyphenol complexes. Their findings imply that these two amino acids having higher affinity with protein (peptide)-polyphenol complexes are found in abundance where a smaller concentration of protein (peptide)-polyphenol complexes is found,

and vice versa. The role of protein (peptide)-polyphenol complexes in regulating N mineralization will be further discussed later.

The three forests that stood out in the PCA biplot had a faster decay constant than the other forests. The attenuating pattern of common amino acids along PC1 suggests that PC1 reflects the balance between supply and consumption (mineralization) of amino acids. Free amino acids are continuously released from the decomposition of organic matter into soils and released amino acids are successively converted to NH_4^+ and NO_3^- in the downstream mineralization pathway. Jones and Kielland (2002) suggested that the pool of free amino acids in soil solution is extremely transient and turns over approximately 20 times per day. The pool of our amino acids must be also very transient. Greater amino acids in the three forests (two lowland forests and Quaternary montane forest) indicate that the supply of amino acids is relatively faster compared to mineralization rate in these forests.

Our results imply that faster N mineralization rates in the three forests (the two lowland forests and Quaternary montane forest) are associated with a faster supply of amino acids which are a precursor of the downstream mineralization pathway. Conversely, slower N mineralization in upland forests can be explained by the limited supply of amino acids for the downstream mineralization. In line with the latter implication, Jones & Kielland (2002) suggested that the transformation of proteins to amino acids, but not amino acids to NH_4^+, was limiting N availability in a taiga forest. Later in an incubation study, Jones & Kielland (2012) further demonstrated that the transformation of high molecular weight dissolved organic N to low molecular weight dissolved organic N limited N mineralization rate in a taiga forest.

Quotient of TNAA to NH_4^+ (Table 2) corroborates the above possibility. Quotient of TNAA to NH_4^+ indicates the ratio of mineralization precursor to the product. Quotient was greater than 0.6 in the three forests where soil N mineralization was fast, while they were <0.29 in the upland forests where soil N mineralization was extremely slow (Table 1), reinforcing our earlier suggestion that the limited supply of amino acids is a bottleneck for the downstream mineralization in the upland forests. Here, a caution is needed because this suggestion assumes that nitrification potential is constant across sites. Hall et al. (2004) assayed nitrification potential of our study sites by adding a saturated amount of NH_4^+ to soil slurry as the substrate for nitrification, and found that nitrification potential actually varied greatly depending on geology and altitude with greater values at lower altitudes and on sedimentary substrate. However, the ultrabasic lowland forest with the highest quotient of TNAA to NH_4^+ demonstrated one of the lowest nitrification potentials, implying that the possibility of overestimating the quotient in the three fast mineralization sites can be ruled out. Effects of plant absorption of NH_4^+ may also affect the interpretation of our results, which needs to be studied in future.

Our NH_4^+ concentration may have been underestimated because our water extraction could not extract those NH_4^+ which were bound with soil cation exchange sites. Therefore, actual quotient of TNAA to NH_4^+ could be much lower, suggesting an even stronger limitation of amino acid supply for the downstream N mineralization pathway. Our TNAA ranged from 0.25 to 7.93 μg/g and these values are comparable with that of Arctic tundra soils. Kielland (1995) reported TNAA values ranging from 2.19 to 8.29 μg/g and NH_4^+ ranging from 0.34 to 0.84 μg/g in water extracts of arctic tundra soils. Weintraub & Schimel (2005) also reported similar TNAA values in water extracts of Alaskan Arctic tundra soils. However, these Arctic tundra soils show much higher quotients of TNAA to NH_4^+ (Kielland, 1995; Weintraub & Schimel

2005), suggesting that mineralization of free amino acids to NH_4^+ is faster or the nitrification (i.e. consumption of NH_4^+) is slower in our tropical soils than in tundra soils.

Yu et al. (2002) found that 48–74% of dissolved organic N in soil leachates of temperate conifer forests in north California consisted of proteins and peptides, most of which were in the form of a hydrophobic fraction. The presence of the hydrophobic fraction indicated that a large portion of dissolved organic N is in the form of protein (peptide)-polyphenol complexes or amino compounds bound with humic substances, which are extremely resistant to microbial proteolysis (Majuakim & Kitayama, 2013). Majuakim & Kitayama (2013) found a greater concentration of a hydrophobic fraction in soil water associated with bound proteins (peptides) under coniferous trees in one of our sites (i.e. the 1700-m sedimentary montane forest, PHQ). They suggested that the bulk of the hydrophobic fraction consisted of protein (peptide)-polyphenol complexes that were resistant to microbial decay. Interestingly, the concentration of protein (peptide)-polyphenol complexes in soil water of the Quaternary montane forest was much lower than the 1700-m sedimentary montane forest in their study. These results suggest that the proteolysis of dissolved organic N is retarded due to the presence of protein (peptide)-polyphenol complexes in the upland forests where TNAA was lower. Conversely, the proteolysis is probably enhanced in soil water due to the absence or lower concentration of protein (peptide)-polyphenol complexes in the three forests where TNAA was higher.

In our study, glutamic acids, aspartic acid, histidine, alanine, cysteine, serine, and phenylalanine were among the most frequently found free amino acids in water extracts of tropical rain forest soils. The following free amino acids were found to be most abundant in high latitude ecosystems in earlier studies: serine, glutamic acid, leucine, ornithine, alanine, aspartic acid and methylamine in Oa horizon leachates of temperate forests in north California (Yu et al., 2002); glycine, aspartic acid, glutamic acid, serine and arginine in water extracts of Arctic tundra soils (Kielland, 1995); serine, glycine, arginine, threonine, and alanine in water extracts of Alaskan Arctic tundra soils (Weintraub & Schimel, 2005); and, glutamine, glutamic acid, alanine, glycine, asparagine and serine in 4mM $CaCl_2$ extracts of temperate forest soils of Michigan, USA (top six amino acids in mole%; Rothstein, 2009). The latter temperate forest soils in Michigan demonstrated significant seasonal and among-site variations in free amino acids (Rothstein, 2009). It appears that water extracts of our tropical forest soils have a similar profile of free amino acids with that of high latitude ecosystems except for histidine and cysteine which are locally abundant in Quaternary montane forests only. However, any conclusion on amino acid profile is premature at this stage due to the very limited data availability. Fractions of high-molecular organic acids in soil water, the rate of their proteolysis, amino-acid mineralization and soil microbial flora must be intricately related to the profile of free amino acids which are extremely transient.

CONCLUSION

As we have demonstrated here, the pool of free amino acids in soil water spatially varies in composition and total N on Mount Kinabalu. Our results imply that slow proteolysis of dissolved organic N in soil water limits N mineralization in upland tropical forests on Mount Kinabalu, which needs to be substantiated in future. Their dynamics must be further studied in relation to protein (peptide)-polyphenol complexes to understand the mechanisms regulating N availability in the context of a proteolysis-mineralization pathway in tropical rain forests.

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DECLARATIONS

Research permit(s). Not applicable. This study was conducted as a joint EcoKinabalu Project with Sabah Parks. Access to the conservation area was granted by Sabah Parks under mutual understanding by both parties.
Ethical approval/statement. Not applicable.
Generative AI use. We declare that generative AI was not used in this study nor in the writing of this article.
Competing interests. The authors declare no conflict of interest, financial or personal, which may potentially influence the work presented in this paper.
Author contributions: KK conceptualized research, raised funds, conducted fieldwork, and wrote the first draft. KK and LM conducted chemical analysis, analyzed data, discussed and finalized draft.

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Supplementary material 1

Appendix I
Authors: Kanehiro KITAYAMA and Luiza MAJUAKIM
Data Type: PDF
Explanation note: List of amino acids analyzed in this study.
Link: https://doi.org/10.51200/jtbc.v23i.7296.g4660

Supplementary material 2

Appendix II
Authors: Kanehiro KITAYAMA and Luiza MAJUAKIM
Data Type: PDF
Explanation note: Concentrations of extractable N as amino acids (μg/g-dry soil) and extractable NH_4^+-N, and quotient of total amino acid-N to NH_4^+-N in soil water extracts of Mount Kinabalu. Sample IDs denoted with O mean samples from organic horizon, and those with M mean samples from mineral horizons. Ult – Ultrabasic, Sed – Sedimentary.
Link: https://doi.org/10.51200/jtbc.v23i.7296.g4661