Short report

Vertical distribution of tree fine roots in the tephra profile with two buried humic soil layers

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Abstract: Surface humic soils, where fine roots are mainly distributed, can be accidentally buried due to coverage by deposits such as volcanic ash. This buried humic soil may influence the vertical distribution of fine roots because soil organic matter strongly affects soil functions. However, fine root distributions in buried humic soils are little understood. In order to elucidate the effects of buried humic soils on fine root distribution, we investigated fine root biomass and soil characteristics in a soil profile down to 3.3 m with two buried humic soils formed by tephra in Tomakomai, Hokkaido, Japan. In this profile, fine root biomass decreased with soil depth, but increased in buried humic soils that had higher soil total carbon (C) content and higher fine soil ratio than buried nonhumic soils. These results lead us to surmise a preferential development of active fine roots in buried humic soils rich in organic C rather than nonhumic soils.

Keywords: deep soil, fine root biomass, vertical distribution, volcanic ash soil

Abbreviations: Absorbed P, Phosphorous absorption coefficient of soil; C, carbon; C:N ratio, carbon/nitrogen ratio; N, nitrogen; P, phosphorus; Truog-P, Soil extractable phosphorus

Introduction

The biomass and distribution of tree fine roots are important variables for understanding the uptake of water and nutrients by trees (Makita et al. 2020). The distribution and metabolism of fine roots are strongly influenced by physical characteristics of soil, such as soil moisture and bulk density, and chemical characteristics of soil, such as soil pH and nutrient contents (Iversen et al. 2018, Borden et al. 2020, Jia et al. 2021). Together, these soil characteristics affect fine root distribution even in deep soils (Zhao and Gong 2021).

The distribution of fine roots (<2mm in diameter) in deep soil may be influenced by the existence of buried humic soil. Buried humic soil is often formed by lava flows, volcanic deposits, weathered loess and floods covering surface humic soil (Chaopricha and Marin-Spíotta 2014) and is relatively rich in soil organic matter for deep soil (Hobara et al. 2020). Humic volcanic soils possess distinctive properties derived from organic matter, such as high water retention, low bulk density (Takahashi and Dahlgren 2016), and high cation exchange capacity derived from organic matter (Suzuki et al. 2005, Kaneko 2015), as compared with other soil types, which can facilitate or accelerate root growth. Thus, fine root distribution in deep soils with buried humic soil may be different from those without. However, root distribution in soil containing buried humic soil has been little studied.

The objective of this study is to understand the fine root distribution pattern in the volcanic ash soils containing buried humic soils. We calculated the biomass of fine roots and investigated the physical and chemical properties of fine roots and soils in a section consisting of several tephras and buried humic soils. We focused on the distribution of fine roots because they are generally physiologically active, performing activities such as nutrient absorption (Makita et al. 2009).

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Materials and Methods

Study site

The study site was in the Tomakomai Research Forest of Hokkaido University in Tomakomai, Japan (42°70′89.96 N, 141°57′08.11 E; Elevation, 63.3 m level ground; 18 km east of Mt. Tarumae). Tephras from Mt. Tarumae are considered a representative of widespread tephras in Hokkaido during the Holocene and have been used as index tephras in various research fields (Tokui 1989, Yoshimoto et al. 2003), and repeated tephra deposition from Mt. Tarumae is observed in the Tomakomai area. The study site had an outcrop exhibiting a 20 m wide soil profile containing two buried humic soil horizons. This soil profile contained three tephras, namely Ta-a, Ta-b, and Ta-c, which were identified using a reference tephra distribution in Hokkaido (Committee on Nomenclature of Pyroclastic Deposits in Hokkaido 1979). Each tephra layer was subdivided into soil horizons based on soil color. The Ta-a tephra, which had a 0–0.64 m soil depth, consisted of three soil horizons: A (Ta-a A), B (Ta-a B), and C (Ta-a C) (Fig. 1). The Ta-b tephra, which had a 0.64–1.95 m soil depth, was subdivided into four soil horizons: AB (Ta-b AB), C1 (Ta-b C1), C2 (Ta-b C2), and C3 (Ta-b C3), with the C layer further divided into three horizons based on the average size of pumice gravel in the soil, from smaller (Ta-b C1) to larger (Ta-b C3) pumice size (Table 1). The Ta-c tephra, which had a 1.95–3.30 m soil depth, was subdivided into A (Ta-c A), B (Ta-c B), and C (Ta-c C) horizons.

The mean annual temperature and total annual rainfall in Tomakomai in 2019 were 8.2°C and 1217 mm, respectively (Japan Meteorological Agency 2020). This site is a secondary forest that naturally regenerated after a typhoon disturbance in 2004, consisting mainly of deciduous broad-leaved trees such as lime tree (*Tilia japonica*), Japanese oak (*Quercus crispula*), and Japanese cucumber tree (*Magonolia obovate*). A Japanese privet (*Ligustrum tschonoskii*) tree was closest (<1 m) to the soil profile investigated and understory vegetation dominated by crown wood-fern (*Dryopteris crassirhizoma*).

In June 2019, we established a sampling area around the 20 m wide outcrop, where we can access intact roots and soils in buried soils with small excavation. A fresh soil section, 3.3 m deep and 5 m wide, was exposed by an excavator car. This soil section contained three tephra layers (Fig. 1). The tephra layers were subdivided into soil horizons based on soil color, and we found humic soil horizons of A or AB for all three tephras.

Sample collection

Fine roots and soils were sampled from each of the ten soil horizons. We selected a sampling location at the center of the excavated soil section, which had representative conditions of vegetation without disturbance and was at least approximately 1 m away from the nearby tree trunks. To measure fine root biomass, soil (including fine roots) was excavated vertically from the ground surface using a stainless-steel 2 L rectangular core sampler (10 cm wide, 10 cm deep, and 20 cm tall, custom-made product). One soil core sample (including fine roots) was taken from each of the 10 soil horizons by inserting a stainless-steel plate between the interface of soil horizons for separation. After collection, the soil cores (including fine roots) were packed in polyethylene bags, stored in a cooling box, and transported to the laboratory. To separate the fine roots and soils, the soil core sample was flooded with tap water in a bucket, stirred, and then filtered first with a 2.0 mm mesh sieve, and second with a 0.71 mm mesh sieve. We used a 0.71 mm mesh sieve to facilitate distinction between roots and soil. After sieving, roots were collected from both what passed through the mesh and what did not pass through the mesh using tweezers. We removed understory fine roots with the reference samples collected from the upper soils of the soil profile. In this study, we defined living and dead fine roots based on the color of the fine root cross-sections, the bending or breaking of the roots when pinched with tweezers, and epidermal peeling (Persson 1983).

Soil samples were collected separately from the fine root core samples. Approximately 2 kg of soil was collected from each horizon using a shovel for chemical analyses. In addition, soil core sample (including fine roots) was taken from each of the ten soil horizons using a 100 mL stainless-steel core sample cylinder (DIK-1801, Daiki Rika Kogyo Co. Ltd). After collection, the soil samples were transported same as the root samples.

Root analysis

Washed root samples were divided into two diameter classes (≥2 mm; <2 mm) using a caliper. To determine the total fine root lengths for diameter classes, we cut fine roots using scissors into ~30 mm sections and spread and arranged the fine roots in an acrylic vat (250 mm wide, 200 mm long, 20 mm tall, 1 mm thick bottom plate, 3 mm thick side plate) filled with water. When the fine roots were sticking out of the water surface, they were cut further. Images were captured using a scanner (GT-X980, EPSON). The total fine root length in each of the three diameter

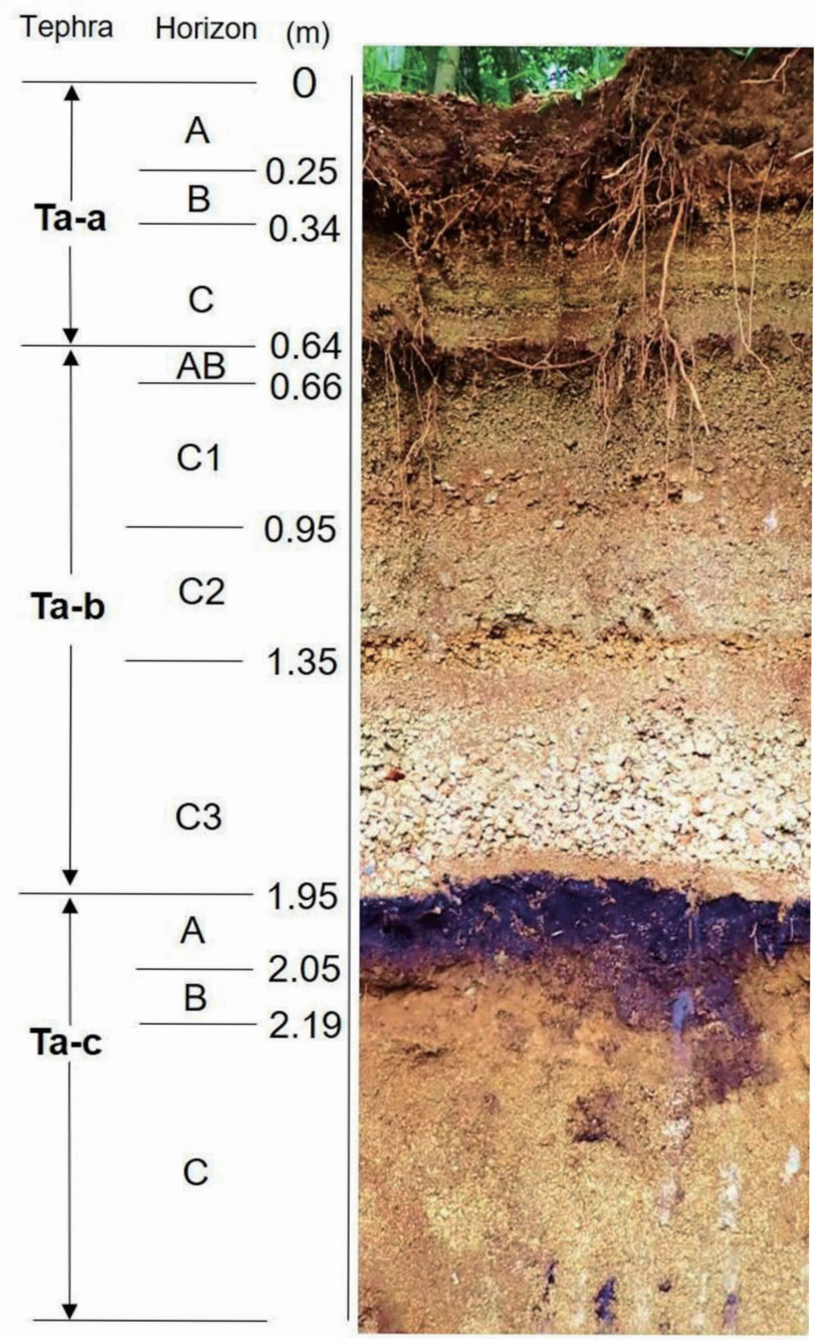

Fig. 1. The soil profile of the study site. Depths (m) from the surface are described for the tephra layers of Ta-a, Ta-b, and Ta-c and the soil horizons (A, AB, B, C, and C1–C3).

classes was calculated from the images using a fine root length analysis macro (Tajima and Kato 2013) in ImageJ: diameter (d) 0.1 mm ≤ d < 0.5 mm, 0.5 mm ≤ d < 1.0 mm, and 1.0 mm ≤ d < 2.0 mm. In root analyses, we did not measure roots with <0.1 mm diameter because these roots seemed to be too thin for woody roots (Ma et al. 2018). After the fine root length analysis, the fine roots were dried at 50°C for more than 72 h to determine the dry weight of fine roots. Dried fine roots were partly powdered and processed for total C and total N content analyses using the combustion method (NC-22F, Sumika Chemical Analysis Service, Ltd.).

Table 1. Fine root total carbon (TC), total nitrogen (TN), and carbon/nitrogen ratio (C:N), of fine roots from each soil tephra and horizon

Tephra	Horizon	TC	TN	TC	TN	C:N
		(g m^{-3})		(%)		
Ta-a	A	1997.3	74.1	41.5	1.6	25.9
	B	1340.6	30.6	43.0	1.0	43.0
	C	27.8	0.4	40.7	0.6	67.8
Ta-b	AB	137.9	3.5	38.7	1.0	38.7
Ta-c	A	40.0	1.2	38.1	1.1	34.6
	B	2.7	0.0	39.0	0.4	97.5

Data for C horizon soils of Ta-b and Ta-c were not shown because fine root samples in these horizons were too small for analyses.

Soil analysis

Soil color and soil texture were determined using standard soil color charts (Oyama and Takehara 2006) and determined soil texture by hand feel flowchart (Yolcubal et al. 2004). The fine soil ratio (diameter, <2 mm) was determined using a mesh screen. The largest gravel size in each soil was measured using a caliper. Soil moisture was determined by oven-drying soil samples (72 h, 50°C). Bulk density was estimated using soil samples taken with the stainless-steel core sample cylinder.

Soil pH (H_2O and KCl) was measured using a pH meter (D-51AC, Horiba) after mixing with ultrapure water (Milli-Q water) and 1 mol L^{-1} KCl, respectively, using a dry soil/solution ratio of 1:2.5. Total soil C and N contents were analyzed same as roots.

Soil N transformation rates were measured using an aerobic laboratory incubation method (Binkley and Hart 1989). The NH_4^+-N and NO_3^--N concentrations in the extract were colorimetrically analyzed using an auto analyzer (Auto Analyzer III type, BLTEC, Inc.). Transformation and nitrification rates were calculated by subtracting the initial concentrations of NH_4^+-N and NO_3^--N for mineralization and NO_3^--N for nitrification from the final concentrations.

Soil extractable phosphorus (Truog-P) was determined using the Truog extraction method (Truog 1930). Soil P concentration was measured with the molybdenum blue method using a spectrophotometer (V-630, JASCO). The P absorption coefficient of soil (Absorbed-P) was measured using the ammonium phosphate method (Nanzyo 1997), and the concentration of P in solutes was evaluated in the same way as that in the Truog-P method.

Results

Fine root distribution and chemical properties

Both total fine root length and fine root weight in the uppermost humic soils (buried humic soils) were

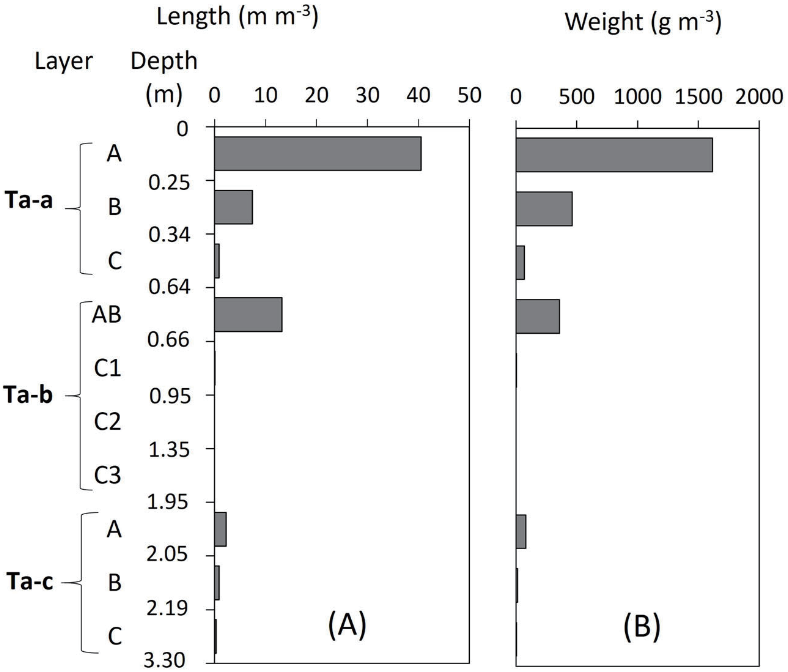

Fig. 2. Total fine root length (A) and total fine root weight (B) for soils in the tephra layers of Ta-a, Ta-b, and Ta-c.

Table 2. Physical characteristics of the study soils

Tephra	Horizon	Depth	Soil color		Fine soil	Largest gravel size	Soil texture	Soil moisture	Soil bulk density
		(m)	JIS notation	Color names	(%)	(mm)		(%)	(kg m^{-3})
Ta-a	A	0–0.25	7.5YR 1.7/1	Brownish black	95.2	10	Sandy loam	34.0	73.3
	B	0.25–0.34	10YR 3/4	Dark yellowish brown	86.9	20	Sand	13.3	83.5
	C	0.34–0.64	10YR 4/6	Yellowish brown	45.7	41	Sand	30.0	88.4
Ta-b	AB	0.64–0.66	7.5YR 2/2	Dark grayish brown	72.7	12	Sand	24.9	93.3
	C1	0.66–0.95	2.5Y 5/4	Brownish olive	24.0	35	Sand	16.4	96.1
	C2	0.95–1.35	10YR 5/3	Grayish brown	55.1	37	Sand	24.4	79.5
	C3	1.35–1.95	10YR 5/4	Yellowish brown	34.4	55	Sand	28.0	47.3
Ta-c	A	1.95–2.05	N 1.5	Black	80.0	5	Silty clay	49.8	53.7
	B	2.05–2.19	7.5YR 3/2	Dark grayish brown	81.7	8	Sandy clay	34.1	95.7
	C	2.19–3.30	2.5Y 4/6	Brownish olive	45.3	38	Sand	18.9	94.0

The JIS is an abbreviation of the Japan Industrial Standards.

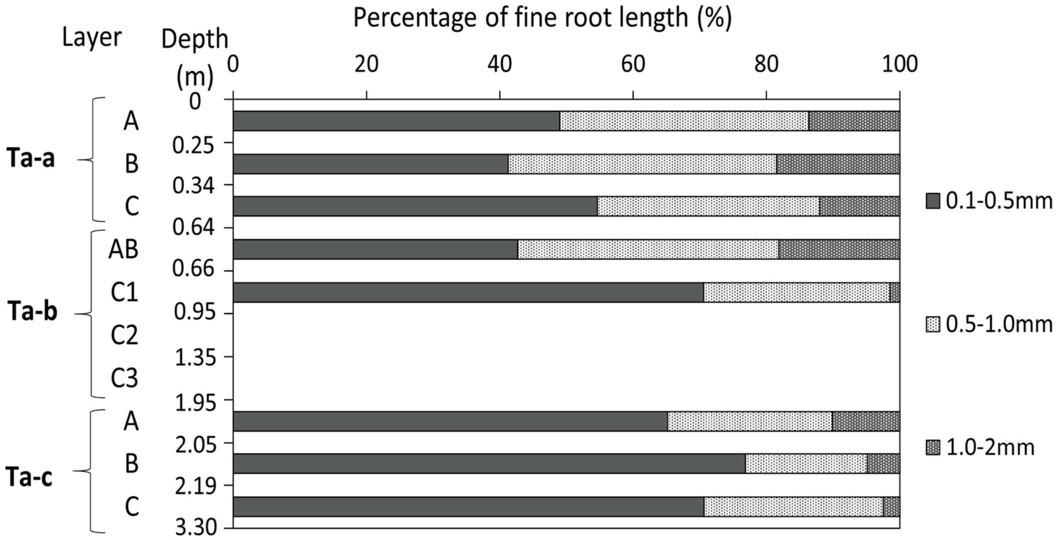

Fig. 3. Percentage of fine root length with three different diameter classes for soils in the tephra layers of Ta-a, Ta-b, and Ta-c.

Table 3. Chemical characteristics of soils in each tephra and horizon: pH (H_2O, KCl), total carbon (TC), total nitrogen (TN), net rate of nitrogen mineralization (N mineralization), absorbed coefficient of phosphorus (Absorbed-P), and soil extractable phosphorus (Truog-P)

Tephra	Horizon	pH		TC	TN	TC	TN	C:N	N mineralization	Absorbed-P	Truog-P
		(H_2O)	(KCl)	(g m^{-3})		(%)			(mg N m^{-3} day^{-1})	(mg P_2O_5 100 g $soil^{-1}$)	(g P_2O_5 m^{-3})
Ta-a	A	5.14	4.17	38106	3.306	5.2	0.5	11.3	12980	1646	10.1
	B	5.50	4.51	7896	0.462	0.9	0.1	17.2	219	1557	24.9
	C	5.89	4.78	3256	0.158	0.4	0.0	22.1	139	1395	17.5
Ta-b	AB	5.98	4.64	15800	0.986	1.7	0.1	16.0	145	1606	7.5
	C1	6.24	5.10	1000	N.D.	0.1	N.D.	N.D.	166	1324	14.6
	C2	6.38	5.23	600	N.D.	0.1	N.D.	N.D.	129	1302	18.2
	C3	6.40	5.21	300	N.D.	0.1	N.D.	N.D.	50	1336	3.9
Ta-c	A	6.12	4.73	27200	1.615	5.0	0.3	16.5	57	2421	4.4
	B	5.94	5.36	10900	0.672	1.0	0.1	16.2	133	2018	8.9
	C	6.00	5.49	2100	0.103	0.2	0.0	25.3	171	1502	6.5

"N.D." means not detected.

greater than those of subsoils of each tephra layers and decreased sharply with soil depth to C horizon soils (Fig. 2). Interestingly, root length and weight varied greatly at the tephra layer boundaries; remarkable increases from Ta-a C (3 m m^{-3}, 68 g m^{-3}) to Ta-b AB (46 m m^{-3}, 357 g m^{-3}) and Ta-b C3 (0 m m^{-3}, 0 g m^{-3}) to Ta-c A (7 m m^{-3}, 82 g m^{-3}) were observed. The highest values for total root length and weight were observed in the uppermost humic soil of Ta-a A (40 m m^{-3}, 1616 g m^{-3}), which were 3.1 and 4.5 times greater than the uppermost humic soil of Ta-b (13 m m^{-3}, 357 g m^{-3}), respectively, and 20.0 and 19.7 times greater than those of Ta-c (2 m m^{-3}, 82 g m^{-3}), respectively. The gravelly C horizon showed small or negligible values for total fine root length and total fine root weight as compared with A, AB, or B soils. The ratio of fine root lengths, Ta-a:Ta-b:Ta-c, in humic soils were 35:12:3. Overall, the total fine root length and weight dropped steeply with increasing soil depth, but marked increases were observed in the humic soils, a pattern that repeated with each tephra through the soil profile.

The percentage of very fine roots (<0.5 mm) in terms of total fine root length was greater than those of fine roots with larger diameters. In the horizons below Ta-b AB, the percentage of fine root length consisting of very fine roots exceeded 60% of the total fine root length (Fig. 3). The lowest percentage of very fine roots in terms of total fine root length was observed at Ta-a B, while the highest percentage was at Ta-c B. The fine root C:N ratio (carbon/nitrogen ratio) was lower in the buried humic horizons than those at gravel horizons (Table 1). The smallest fine root C:N ratio, 25.9, was observed in Ta-a AB, which was followed by Ta-c A (34.6), and Ta-b A (38.7); the largest soil C:N ratio was 97.5 in the Ta-c B. Fine roots in gravelly soils had considerably higher C:N ratios compared to the surface and buried humic horizons.

Physical and chemical soil properties

The physical and chemical properties of soils changed with the horizons and at the tephra boundaries throughout the soil profile. The humic soils such as A and AB horizon (72.7%–95.2%) had higher percentages of fine soil than C horizons (24.0%–55.1%), which contained mostly large gravels (Table 2). Similarly, soil texture was finer in the upper humic A and AB soils of each tephra. Soil moisture was also relatively high in the humic A or AB horizon of each tephra, and the highest soil moisture was measured in Ta-c A. In Ta-a and Ta-b tephras, soil moisture was lowest at the horizon beneath the humic A or AB horizons, whereas the Ta-c tephra had the lowest soil moisture in the bottom horizon. Soil bulk density ranged from 8–10 Mg m^{-3} and was lowest in the humic A or AB horizons in Ta-a and Ta-c and in the C3 horizon in Ta-b.

The pH was acidic in all soils, with lower values for pH (KCl) than pH (H_2O) (Table 3). Within each tephra layer, the buried humic horizon of A and AB had relatively low pH (more acidic) compared with the other soils.

Total soil C content was higher in the buried humic soils (1.7%–5.0%) than in the B and C horizons (0.1%–1.0%). The trend in total soil N was similar to that of total soil C. The soil C:N ratio was lower in the humic A, AB, and B horizon in the Ta-a and Ta-c tephras (11.3–17.2) than the C horizons (22.1–25.3).

The net rate of N mineralization was highest in the Ta-a A at 12,980 mg N m^{-3} d^{-1}, which was three to four orders higher than the other soils. The Ta-a A horizon had the highest N mineralization rate in the Ta-a tephra; however, the top humic soils in the Ta-b and Ta-c tephras did not exhibit high N mineralization rates.

Truog-P was lowest in the humic A and AB horizon for each tephra, while the intermediate horizon below the uppermost humic soils was higher. The Absorbed-P was higher in the humic A or AB horizon within each tephra, decreasing with horizon from A or AB > B > C.

Relationship between soil and fine roots

Within each tephra, greater fine root biomass was observed for upper humic soils with higher organic C content (Fig. 4). Deeper buried humic soils showed lower fine root biomass than upper humic soils for the same total C content.

Discussion

Fine root distribution in the soil profile containing buried humic soils

The main finding of this study is that fine root biomass increased repeatedly at the buried humic soils (Fig. 2). Although in forest soils, fine root distributions typically decrease with soil depth, in this study, the soil profile containing buried humic soils did not show a unidirectional decrease in fine roots with depth, but repeated increases at the buried humic soils. In the soil profile, the percentage of total fine root lengths for very fine roots, which are thought to be highly active (Makita et al. 2009), were greater in the deeper soils in tephra layers than the surface soil (Fig. 3). The fine root C:N ratio was lower in the buried humic soils than the gravel soils (Table 3), which is indicative of a high nutrient absorption activity (Gordon and Jackson 2000). In addition, fine roots are known for preferable distribution in the nutrient-rich environment (Hodge 2004). Therefore, the repeated increases in fine root biomass in buried humic soils lead us to surmise that tree plants prefer humic soil conditions than nonhumic gravelly soil conditions, which may have resulted in further development of fine roots into the buried humic soil.

Possible factors affecting fine root distribution in buried humic soils

The results showed that the fine root biomass was higher in buried humic soils than in nonhumic soils (Fig. 2). Within each tephra, greater fine root biomass was observed for upper humic soils, showing higher organic C (Fig. 4). In addition, deeper buried humic soil showed lower fine root biomass than upper humic soil for the same C value. The relationship between soil total C and fine root

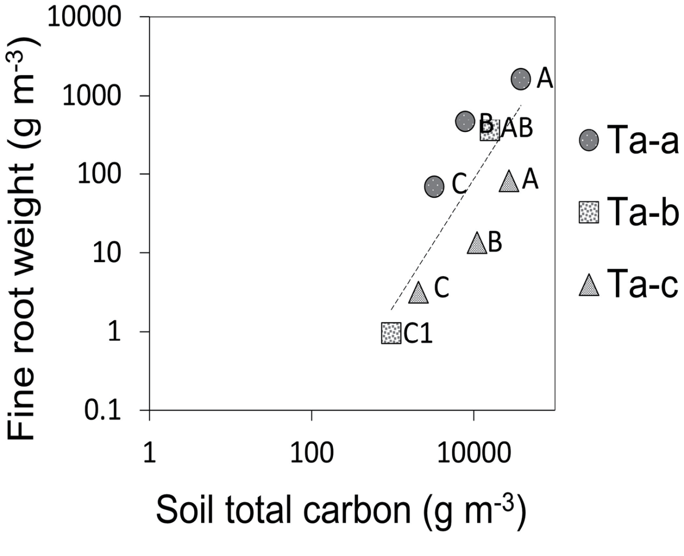

Fig. 4. Relationship between fine root weight and total C in soils. The figure is presented on a logarithmic axis. The solid line indicates the regression line (y=0.00003$x^{1.6243}$, R^2 = 0.6668, P < 0.01). The data of Ta-b C2 and Ta-b C3 horizons are not shown because the total C contents were below the detectable limits.

biomass (Fig. 4) indicates that soil organic matter is a key factor that increases fine root biomass in soils, including buried soils. In addition, lower fine root biomass in deeper buried soil raises the possibility that the influence of soil organic matter on fine root biomass decreases with burial and soil depth. Soil organic matter is a reservoir of many plant nutrients, such as N and P, and provides a substantial cation exchange capacity, which is important for nutrient retention (Powlson et al. 2011). Furthermore, soil organic matter affects soil physical properties. For example, soil organic matter increases water holding capacity (Bhadha et al. 2017), facilitates the formation of stable aggregates that improve aeration, and makes fine root penetration easier (Powlson et al. 2011). Therefore, the abundant soil organic matter in buried soils may have affected fine root distribution, even in deep (>2 m) soils. Nutrients are also considered a potentially important factor for fine root growth. However, nutrient availability was not necessarily high in buried humic soils, as indicated from the results of the N mineralization rate and the Truog-P method, suggesting these influences are small.

As described previously, the abundant soil organic matter surmised to affect fine root distribution is associated with volcanic ash deposition. Volcanic ash is an abundant source of active aluminum and iron hydroxide minerals (Dahlgren and Ugolini 1989), and the association of these minerals with humic organic matter results in the formation of soil with low weight, dark color, low bulk density, and high water retention capacity (Brady and Weil 2002). This association is more abundant in finer ash tephra with a larger specific surface area than in gravelly tephra. When volcanic ash falls, this finer ash with low density accumulates on the ground rather than gravelly tephra with high density, resulting in finer ash in the upper layer of the soil profile and gravelly tephra in a lower position (Nanzyo et al. 2017). Furthermore, plant litter provided abundantly to surface soil. Therefore, in buried tephra layer, organic matter is most abundant in the uppermost horizon, forming buried humic soil. These findings suggest that fine roots in soil with multiple tephra layers prefer the upper humic soil condition of each tephra.

Further research

In this study, we focused on clarifying vertical changes in fine root biomass through soil profiles containing three humic layers. However, our experimental design does not seem to warrant the generality of the results. Furthermore, identification of the tree species of fine roots was not performed. Therefore, further research is needed to determine the representative patterns of fine root distribution in such profiles. In this study, the effects of N and P on fine root distribution were not validated, which may also require further investigations. Furthermore, organic matter in buried humic soils is stable against decomposition. Thus, the investigation of the effects of fine roots in buried humic soils on soil organic C sequestrated in deep soils would be beneficial.

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