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MANUSCRIPT

Root exudation and biodegradation of organic acids in a tropical forest soil under dipterocarp and pioneer trees

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Abstract

Aims Root exudation of organic acids is one of strategies for tropical trees to facilitate nutrient uptake from the highly weathered soils. However, paradoxical relationship remains that root exudation also stimulates microbial activities to consume organic acids in the rhizosphere (root-soil interface). Plant-specific root exudation might shape different rhizosphere carbon (C) cycles in soils under different tree species. We test whether root exudation and rhizosphere C fluxes of organic acids and sugars differ between soils under dominant dipterocarp trees (*Dipterocapus cornutus* and *Shorea laevis*) and pioneer trees (*Macaranga* spp.).

Methods We measured (1) root exudation from mature trees, (2) soil solution concentrations of organic acids and monosaccharides, and (3) mineralization kinetics of ^{14}C-radiolabelled substrates in the rhizosphere and bulk soils of the Dipterocarp and Macaranga trees.

Results Malate was a dominant organic acid exuded from Dipterocarp roots, while monosaccharides were dominant exudates of pioneer Macaranga trees. Malate exudation rates by Dipterocarp roots were greater compared to Macaranga roots. Organic acid exudation increased with increasing root surface area and with decreasing soil pH. Microbial activities of malate mineralization were enhanced in the rhizosphere both under Dipterocarp and Macaranga trees, but the C fluxes of malate mineralization far exceeded root exudation of malate in the rhizosphere of Dipterocarp trees.

Conclusion Tree species develop different strategies to increase malate concentration in rhizosphere soil directly through root exudation or indirectly through rhizosphere microbial activities to increase malate production, which might be favorable for phosphorus solubilization, aluminum detoxification, and lignin degradation in acidic soils.

Keywords Decomposition · Organic acid · Phosphorus deficiency · Sorption · Tropical forest

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Introduction

In the tropical forests, long-term weathering and acidification of soils generally result in a decrease in available phosphorus (P) (Walker and Syers, 1976). This is hypothesized to cause a decline in biomass productivity (Wardle et al. 2004), but high productivity could be maintained by plant species diversity and habitat differentiation (Fujii et al. 2018). There should occur a variety of belowground strategies of tropical tree species to acquire nutrients from the highly weathered soils (Fujii et al. 2018; Ma et al. 2018).

The soil in the vicinity of fine roots, termed as rhizosphere, is a hotspot for carbon (C) and nutrient cycles (Kuzyakov and Razavi, 2019). Root exudation of low molecular weight organic acids (LMWOAs) such as oxalic, citric, and malic acids, can increase in response to P deficiency to solubilize recalcitrant P bonded with aluminum or iron oxides (Jones, 1998). Although the concentrations of LMWOAs are generally low in soil solution (Strobel, 2001), they increase towards root surface due to the localized substrate inputs (Jones et al. 2004). Root exudation of labile substrates increases the biomass and/or activity of rhizosphere microorganisms and promotes nutrient cycles through priming effects (Dijkstra et al. 2013). However, there was a paradox that root exudation increases microbial activity to consume exuded LMWOAs in the rhizosphere (Fujii et al. 2013). Organic acids can also be consumed through sorption onto the solid phase (Ström et al. 2001). Mineralization and sorption risk under/overestimating the efficacy of the exuded LMWOAs in rhizosphere processes (Jones et al. 2003).

Diverse plant roots release different kinds and amounts of low molecular weight organic substances (LMWOSs; LMWOAs and sugars) in tropical forests (Grayston et al. 1996; Aoki et al. 2012). The decomposability of LMWOS and extent of sorption are compound-specific (Jones and Brassington, 1998; Ström et al. 2001; Van Hees et al. 2003). Microbial community grown in presence/absence of LMWOAs tends to increase/decrease transporter activity and dependency of LMWOAs for microbial respiration (Jones et al. 1996; Fujii et al. 2019). When a certain tree species releases specific root exudates, rhizosphere microbes might induce a shift towards specializer of root exudates and re-shape rhizosphere C and nutrient cycles. The importance of these direct and indirect effects in rhizosphere or whole soil C cycles has rarely been quantified.

Bornean primary forest accommodates dominant species (*Dipterocapus cornutus* and *Shorea laevis*) and pioneer species (*Macaranga gigantea*) that differ in terms of root morphology (finer roots vs. coarser roots) and ecology (ectomycorrhizal roots vs. arbuscular mycorrhizal roots) (Smits, 1994). The greater aboveground biomass and longer lifespan of dipterocarp trees, compared to Macaranga trees, should require greater amounts of organic acid exudation to solubilize P and detoxify Al^{3+} and have mechanisms to maintain high levels of organic acids in the rhizosphere. We hypothesized that root exudation and rhizosphere LMWOS-C fluxes could differ between contrasting tree species.

Here, we tested (1) whether composition and amounts of root exudation differ between dominant dipterocarp trees and pioneer trees, (2) whether different root exudates cause specialization of substrate mineralization by rhizosphere microbes, and (3) whether root exudation and rhizosphere C fluxes are quantitatively important relative to the bulk soil C cycles.

Materials and Methods

Site description

Experiments were carried out in tropical forests and agroecosystems in Bukit Soeharto (S0°51', E117°06'; 99 m a. s. l., average inclination 15°), East Kalimantan Province, Indonesia (Fig. 1). After the fires in 1982–1983, we established five plots (20 m × 20 m) of undisturbed primary dipterocarp forest dominated by *Dipterocapus cornutus* and *Shorea laevis* and natural secondary forest with regeneration by pioneer species *Macaranga gigantea*, respectively. The stand age of Macaranga forests are ca. 20 years old after the 1998 fires (Slik et al. 2003). The mean annual air temperature was 26.8ºC, and the mean annual precipitation was recorded as 2187 mm yr^{-1}. Soils were derived from sedimentary rocks and classified as Typic Paleudults (Soil Survey Staff, 2014). The detailed features of these sites and soil properties are described in Fujii et al. (2020a).

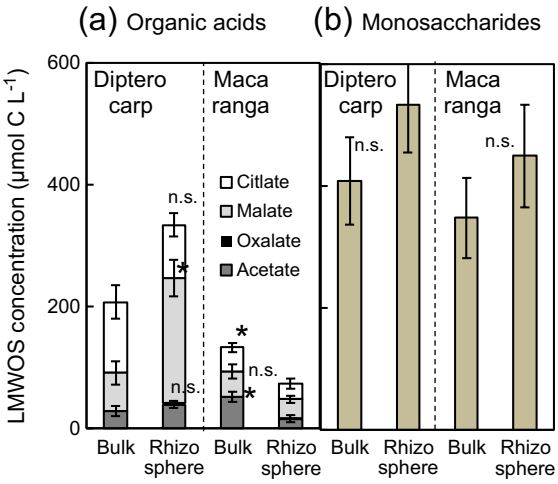

Fig. 1 Concentrations of (**a**) low molecular weight organic acids and (**b**) monosaccharides in rhizosphere and bulk soil solutions. Bars indicate standard errors ($N=5$). The statistical significance of differences between rhizosphere and bulk soil solutions was indicated by * ($P<0.05$) or n.s. (not significant)

Soil sampling

The composite soil samples were collected from three pits at each plot in August 2012. The distance between each pit was 10 m. The surface mineral soil horizons (A horizon; 0–10 cm), where roots and microbial activities are considered to be high, were collected and analyzed (Table 1). As previously outlined in Fujii et al. (2013), the unsieved fresh soil samples were separated into rhizosphere and bulk fractions. Rhizosphere soil fractions were obtained by collecting the soil materials adhering closely to roots, after gently shaking the fine root systems (diameter < 2 mm) 10 times until the loosely adherent soil was removed. The bulk fractions were collected from the soil outside the rooting area. After removing rhizosphere soil, roots were washed and scanned in a flat screen scanner (GT-S600, EPSON, Tokyo, Japan), and the root surface area and root tip number were analyzed using Win-Rhizo (Regents Instruments Inc., Quebec, Canada). The thickness of the rhizosphere soil was calculated by dividing the volume of the adherent soil by the root surface area. The percentage of the rhizosphere soil relative to the total mass of soil was calculated from the thickness of the rhizosphere soil, root surface area, and bulk density.

Table 1 Aboveground biomass and physicochemical properties of tropical forest soils

Site	Aboveground biomass	Fine root biomass[a]	pH		Total C[b]	Total N[b]	Available P[b]	Microbial biomass C[b]	Particle size distribution[bc]		
									Sand	Silt	Clay
	(Mg ha^{-1})		H2O	KCl	(g kg^{-1})		(mg kg^{-1})	(mg C kg^{-1})	(%)		
Dipterocarp	286.3	3.0 ± 0.3 A	4.1 ± 0.1 B	3.6 ± 01B	26.5 ± 0.1	1.9 ± 0.1	9.3 ± 1.2B	207 ± 23 A	52 ± 2	25 ± 1	23 ± 2
Macaranga	47.3	1.8 ± 0.2B	4.7 ± 0.1 A	4.3 ± 0.1 A	31.7 ± 0.2	2.0 ± 0.3	20.3 ± 2.5 A	179 ± 25A	50 ± 1	25 ± 1	25 ± 1

[a]The fine root biomass in the 0–10 cm soil was measured. Within each column of each site, different letters (A, B) indicate that values are significantly ($P<0.05$) different between sites

[b]The results are expressed on an oven-dry (105 °C, 24 h) weight basis. Mean ± standard errors ($N=5$)

[c]Clay (< 0.002 mm); Silt (0.002–0.05 mm); Sand (0.050–2 mm)

Physicochemical analysis of soils

A proportion of the fresh soil samples were air-dried and sieved (< 2 mm) to eliminate litter, roots and pebbles. Soil pH was measured using a soil to solution (H_2O or 1 M KCl) ratio of 1:5 (w/v) after shaking for 1 h. Total C and N concentrations were measured using a CN analyzer (Vario Max CN, Elementar Analysensysteme GmbH). Particle size distribution [clay (< 0.002 mm); silt (0.002–0.05 mm); sand (0.050–2 mm)] was determined using the standard sedimentation method. The available phosphorus (P) concentrations were estimated using the Bray 2 extraction method (Blakemore et al. 1987). The microbial biomasses C was determined using the chloroform fumigation-extraction method (Vance et al. 1987) with conversion factor of 0.45 (Wu et al. 1990). The soluble C in the fumigated and non-fumigated soil samples were extracted with 0.5 M K_2SO_4 (soil to solution ratio of 1:5) and measured using a total organic C analyzer (TOC-V CSH; Shimadzu, Japan).

Soil solution extraction and chemical analysis

The centrifugation-drainage technique was used to extract soil solution (Giesler and Lundström, 1993). Without addition of water, the rhizosphere and bulk soil fractions were centrifuged for 30 min at a speed of 8,800 rpm (10,560 g; ~ 1.5 MPa; Hitachi centrifuge) within 36 h of sampling, respectively. The soil solution extracts were filtered through a 0.6 µm filter (GF/C, Whatman) and frozen at -24ºC prior to analysis. The monosaccharide concentrations were determined using periodate oxidation (Burney and Sieburth, 1977; Johnson and Sieburth, 1977) and glucose standards. The concentrations of LMWOAs were determined by high performance liquid chromatography (HPLC, Shimadzu, Japan) using the method by Van Hees et al. (1999). Organic acids were separated on a Supelcogel C610-H ion exclusion column using 0.1% H_3PO_4 as the mobile phase at operating temperatures of 60ºC for citric acid and 30ºC for oxalic and malic acids with UV detection at 210 nm.

Organic acid and glucose mineralization kinetics

As previously outlined in our previous study (Fujii et al. 2019), C fluxes of LMWOS in soils were estimated for the rhizosphere and bulk soil fractions. ^{14}C-radiolabelled glucose or organic acid solution (100 µL; specific activity: 0.17 kBq mL^{-1}; pH 4.5) was added to 1 ± 0.02 g of field-moist soil in 50-mL polypropylene vials. ^{14}C-glucose (U-^{14}C; American Radiolabeled Chemicals, Inc., 0.4 GBq mmol^{-1}) and four organic acids, ^{14}C-acetic acid (1,2-^{14}C; 2.2 GBq mmol^{-1}), ^{14}C-oxalic acid (1,2-^{14}C; 0.2 GBq mmol^{-1}), ^{14}C-malic acid (1,2-^{14}C; 0.2 GBq mmol^{-1}), and ^{14}C-citric acid (1,5-^{14}C; 2.2 GBq mmol^{-1}), were used in the mineralization assays. The initial solution concentrations of each substrate were 50, 250, 500, and 1000 µM. Following addition, the soil was gently shaken to ensure mixing and incubated at 25 °C in sealed vials. ^{14}C-CO_2 produced by mineralization of the added substrate was collected in a plastic scintillation vial containing 1.0 mL of 1 M NaOH placed on top of the soil, separated by a spacer. The ^{14}C-CO_2 concentrations trapped in NaOH were determined by liquid scintillation using alkali-compatible scintillation fluid (Hionic-Fluor; Perkin Elmer). ^{14}C-CO_2 production was measured during the initial linear phase of decomposition (1 h), which was confirmed by the pilot experiment.

The data of mineralization kinetics were fitted to a single Michaelis–Menten equation:

$$V = \left(V_{max} \times C\right) / \left(K_M + C\right) \quad (1)$$

where V is the mineralization rate (nmol g^{-1} h^{-1}), C is the substrate concentration (µM) in the soil solution, V_{max} is the maximum mineralization rate (nmol g^{-1} h^{-1}), and K_M is the concentration at which the half-maximal mineralization rate occurs $\left(1/2\, V_{max}; \mu M\right)$. Michaelis–Menten plots of organic acids were constructed using the equilibrium organic acid concentrations in soil solution, assuming complete mixing of the organic acid with the intrinsic soil water and the sorption reaction (see the following section).

Sorption isotherms of organic acids

Glucose is not adsorbed onto the solid phase due to a lack of charge, whereas negatively charged carboxylic acids (acetic, oxalic, malic, and citric acids) are strongly adsorbed onto the solid phase (Jones and Brassington, 1998). To estimate the equilibrium

concentrations of the organic acids in the soil solution after adding organic acid in the kinetic experiments, sorption isotherms were obtained by the method of Fujii et al. (2019). In each tube, 2.5 mL of ^{14}C-radiolabelled organic acid solution (170 Bq mL^{-1}; pH 4.5) was added to 0.50 g of chloroform-fumigated (48 h) field-moist soil in 6-mL plastic vials with a soil to solution ratio of 1:5 (w/v). The initial organic acid concentration ranged from 100 to 1000 µM. Following addition, the samples were shaken for 10 min on a reciprocating shaker at 320 rpm. The samples were subsequently centrifuged (16,000 × *g* for 5 min) and the supernatant was recovered. The equilibrium ^{14}C concentrations in solution were determined by liquid scintillation counting (Aloka Liquid Scintillation System, LSC-3050; Hitachi) using Optiphase HiSafe 2 scintillation fluid (Perkin Elmer, Japan).

Then, the sorption isotherm data were fitted to the Freundlich equation:

$$A = k \times C^{1/n} \quad (2)$$

where A is the quantity of organic acid adsorbed (nmol g^{-1}), C is the equilibrium solution concentration (µM), k is the Freundlich's constant related to soil ability to sorb organic acid, and $1/n$ is the constant related to sorption intensity. The quantity of anion adsorbed (A) can be calculated using the following equation:

$$C_{tot} = (C \times \theta) + (A \times \gamma) \quad (3)$$

where C_{tot} is the total quantity of organic acid added to the soil (nmol cm^{-3}), C is the equilibrium soil solution concentration (µM), θ is the volumetric water content (cm^3 cm^{-3}), and γ is the soil bulk density (g cm^{-3}).

Rhizosphere effects of substrate mineralization rates

The mineralization rates of LMWOSs at their actual substrate availability were estimated using Eq. 1, assuming that LMWOSs in soil solutions are utilized by soil microbes as described by Michaelis–Menten kinetics. Using the bulk density, the volumes of the rhizosphere and bulk soil fractions, the mineralization rates were scaled up to the C fluxes in the surface soil horizon (0–10 cm). The rhizosphere effects (R) were assessed by dividing the rhizosphere C flux fraction [$F_{\mathrm{Rhizosphere}}$, F_{Bulk} (mmol C m^{-2} h^{-1})] relative to rhizosphere soil mass fraction [$M_{\mathrm{Rhizosphere}}$, M_{Bulk} (%)].

$$R = (F_{Rhizosphere}/F_{Bulk})/(M_{Rhizosphere}/M_{Bulk}) \quad (4)$$

Quantification of root exudation rates

Root exudates were collected from alive roots of mature trees, according to Phillips et al. (2008). We selected two dominant species (*Dipterocapus cornutus* and *Shorea laevis*) in primary dipterocarp forest and one pioneer species (*Macaranga gigantea*) in Macaranga forest, respectively. The alive fine root systems (diameter < 2 mm) were carefully excavated from the soil at the boundary between the organic and mineral soil horizons. After the roots were carefully rinsed with distilled water to remove the adhering soil, the alive root systems were placed in 50 mL syringes filled with sterile acid-washed glass beads and a C-free solution. After 48 h, the solution containing exudates was collected using another syringe. The experiments were conducted with five replicates for each species. We selected one root system from a single tree and performed an exudation experiment. The five trees were examined for each tree species. The solutions were filtered through a 0.6 µm filter (GF/C, Whatman) and frozen at –24ºC prior to analysis. The concentrations of monosaccharides and acetic, oxalic, citric, and malic acids in the solutions were determined as with soil solution analyses. Rates of root exudation (nmol g^{-1} root h^{-1}) were calculated using the organic acid concentrations (nmol L^{-1}), the quantity of solution in the syringe (30 mL), and the time between sample collections (48 h). Root exudation measured in this method corresponds to net exudation from plant roots and mycrorrhizal hyphae, which underestimates the gross root exudation due to mycorrhizal C consumption but quantifies C inputs release into rhizosphere from plant roots and mycrorrhizal hyphae.

Calculations and statistics

All results are expressed on an oven-dry (105 °C, 24 h for soil and 70 °C, 48 h for root) weight basis and are the mean ± standard error (SE) of five replicates. The statistical differences of mean values between groups (tree species, rhizosphere vs. bulk soil fraction) were

tested using analysis of variance (ANOVA) at a $P < 0.05$ significance level for soil solution concentrations, sorption isotherm parameters, mineralization kinetics parameters, and root exudation rates. The statistical analyses were performed using SigmaPlot 14.0 (SPSS Inc., 2020). The Michaelis–Menten equation and Freundlich equation were fitted to the mineralization kinetics data and the sorption isotherm data, respectively, using a least-squares optimization procedure with SigmaPlot 14.0. The significant differences of the Michaelis–Menten and Langmuir parameters were tested with a modified *t*- test and the modified Tukey method (Zar, 1999).

Results

Soil and root properties of Dipterocarp and Macaranga forests

Under the Macaranga trees, soil pH and available P were higher than under the Dipterocarp trees due to the ash inputs in the past fires (Table 1). There was no difference in microbial biomass C between Dipterocarp and Macaranga forest sites (Table 1). Although Dipterocarp tree roots had larger root surface areas and more root tips (Table 2), the rhizosphere soil was thinner in the Dipterocarp roots (avg. 1.3 mm) than in the Macaranga roots (avg. 3.5 mm). The rhizosphere soil fraction constituted 5.5% and 8.3% of the total soil masses in the Dipterocarp and Macaranga sites, respectively.

Low molecular weight organic substance concentration in rhizosphere and root exudation

Compared to the bulk soil, rhizosphere soil solution displayed significantly higher concentrations of malic and oxalic acids in the Dipterocarp soil (Fig. S1; Fig. 1). No enrichment effects of LMWOAs in the rhizosphere were observed in Macaranga soil (Fig. S1; Fig. 1). There was no significant difference in acetic acid and monosaccharide concentrations in rhizosphere and bulk soil solutions between Dipterocarp and Macaranga sites (Fig. S1; Fig. 1).

Monosaccharides, acetic, malic, and citric acids were detected in root exudates, but the composition and rates differed between Dipterocarp trees (*Dipterocapus cornutus* and *Shorea laevis*) and Macaranga trees. The organic acid exudation rates were positively correlated with root surface areas (Fig. 2a) and with root tips, respectively. Root exudation rates of malic and acetic acids by the Dipterocarp trees were greater than the Macaranga trees (Table 2). This contrasts with the higher monosaccharide exudation of the Macaranga trees (Table 2). There was no significant difference between *Dipterocapus cornutus* and *Shorea laevis*, except for in oxalic acid exudation (Table 2).

Table 2 Root traits and exudation rates of Dipterocarp and Macaranga trees

Site / Tree species	Root tip number	Specific root surface area	Root exudation rate					Root exudation C flux	
			Mono saccharides	Acetate	Oxalate	Malate	Citrate	$_{Total}$	Ox + Mal + Cit
	(g^{-1} root)	($cm^2\ g^{-1}$ root)	(nmol $h^{-1}\ g^{-1}$ root)					(mol C m^{-2} $month^{-1}$)	
Dipterocarp									
Dipterocarpus cornutus	21,641 ± 1200 A	539 ± 157 A	238 ± 54 B	387 ± 261 A	0 ± 0 B	261 ± 33 A	320 ± 99 A	11.3 ± 1.6A	6.1 ± 0.9A
Shorea laevis	13,890 ± 3422 A	318 ± 16 A	264 ± 60 B	576 ± 154 A	1 ± 0 A	215 ± 117 A	267 ± 64 A	267 ± 64 A	4.8 ± 1.2 A
Macaranga									
Macaranga gigantea	2049 ± 1373 B	155 ± 51 B	468 ± 41A	40 ± 10B	O ± OB	O ± OB	202 ± 60 A	7.3 ± 0.8 A	2.1 ± 0.5 B

Mean ± standard errors ($N = 5$). Within each column of each site, different letters (A, B) indicate that values are significantly ($P < 0.05$) different between tree species

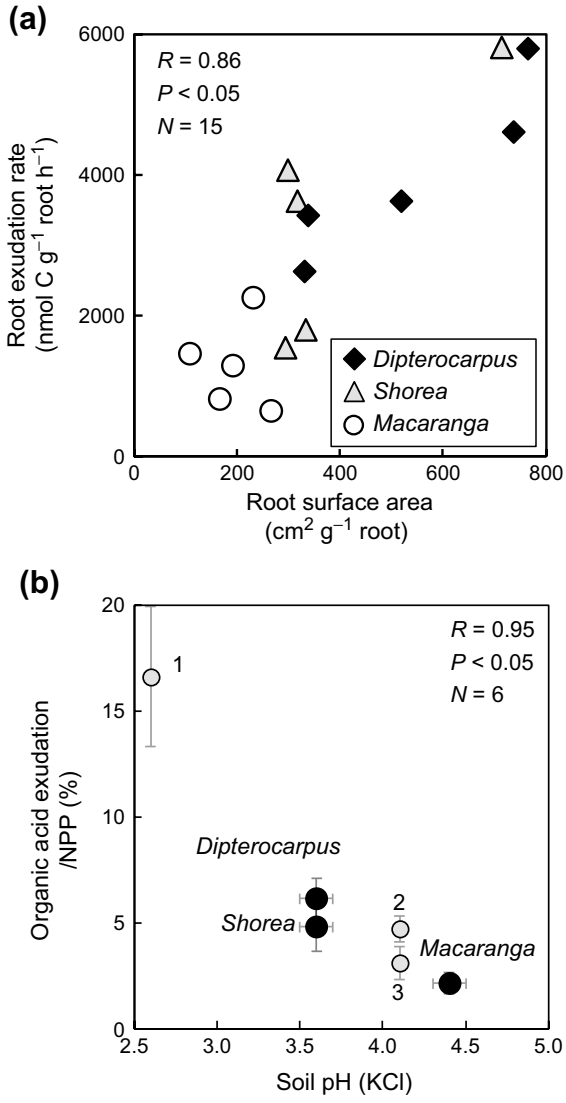

Fig. 2 **a** Relationship between root surface area and root exudation rate of organic acids, (**b**) relationship between root exudation of organic acids relative to net primary production (NPP) and soil pH (KCl). Acetate, oxalate, malate, and citrate were counted in Fig. 2a, while oxalate, malate, and citrate were counted in Fig. 2b to compare with the previous study [1, 2 = tropical montane forest, 3 = tropical forest (Aoki et al. 2012)]. Bars indicate standard errors ($N = 5$)

Using the exudation rates and the fine root biomass in the soil profile (0–10 cm; Tables 1 and 2), the C fluxes of root exudation rates were roughly estimated for Dipterocarp and Macaranga forests (Table 4). In the Dipterocarp and Macaranga forests, the C fluxes of multivalent organic acid (citric, malic, and oxalic acids) exudation corresponded to 4.8–6.1% and 2.1% of net primary production (NPP) [5.1 mol C m^{-2} $month^{-1}$ (Toma et al. 2000) and 6.2 mol C m^{-2} $month^{-1}$ (Gamo, 2003), respectively] (Table 2). When the published data (Aoki et al. 2012) and those from the present study were included in correlation analysis, the proportions of organic acid exudation relative to NPP were negatively correlated with soil pH (KCl) (Fig. 2b).

Organic acid sorption reactions

To estimate organic acid sorption in the mineralization kinetics experiment, the data of organic acids sorption and equilibrium concentration were fitted well to a Freundlich equation ($R^2 > 0.95$; Fig. 2; Table 3). Among the four organic acids, the degree of organic acid sorption followed the order: malate > citrate > oxalate > acetate (Fig. 3). There were no differences in sorption of respective organic acids between the soils under Dipterocarp and Macaranga trees (Fig. 3).

Mineralization kinetics of low molecular weight organic substances in the rhizosphere

We compared mineralization kinetics to assess the specialization of substrate mineralization by rhizosphere and bulk soil microbes. The mineralization rates of both soils varied between substrates and followed the order: malate > citrate, oxalate > acetate, glucose (Fig. 4). The higher mineralization activities in the rhizosphere, compared to the bulk soil, were observed for malate and oxalate in the Dipterocarp soil, but only for malate in the Macaranga soil

Table 3 Freundlich isotherm parameters, sorption capacity (*k*) and sorption intensity (*1/n*), to describe organic acid sorption in soil

Mean values ($N = 5$) were presented

Site	Acetate		Oxalate		Malate		Citrate	
	k	*1/n*	*k*	*1/n*	*k*	*1/n*	*k*	*1/n*
	μmol g^{-1})		μmol g^{-1})		μmol g^{-1})		μmol g^{-1})	
Dipterocarp	0.004	0.61	0.002	0.69	O.Dl5	0.71	0.005	0.82
Macaranga	0.001	0.94	0.002	0.73	0.012	0.73	0.003	0.90

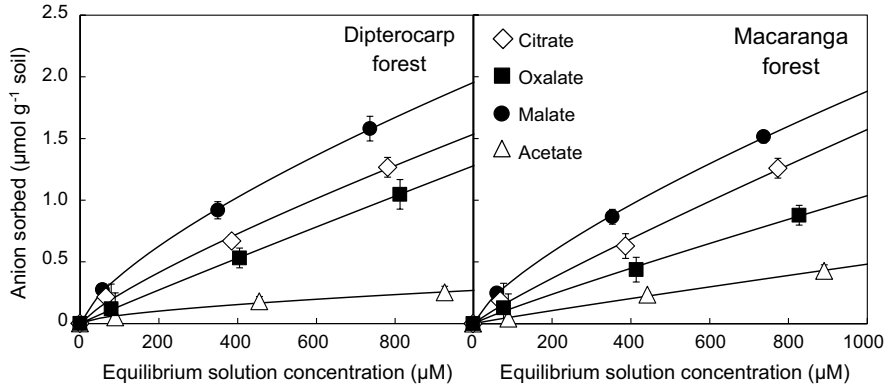

Fig. 3 Concentration-dependent sorption of citrate, oxalate, malate, and acetate in soils. Symbols denote experimental points, while the curves represent Freundlich sorption isotherms fitted to the experimental data. Bars indicate standard errors ($N=5$)

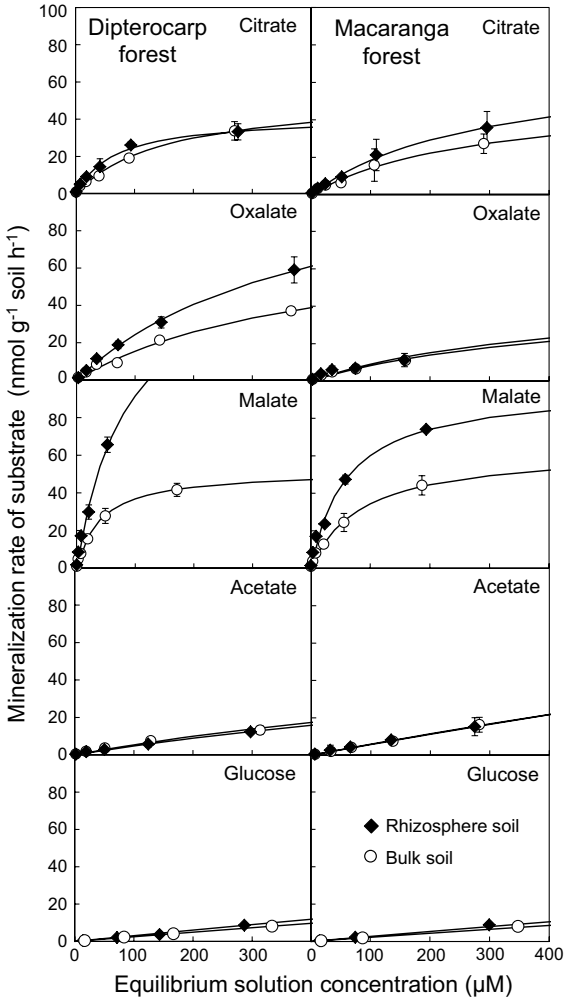

Fig. 4 Concentration-dependent mineralization of citrate, oxalate, malate, acetate, and glucose in soils. Symbols denote experimental points, while the curves represent Michaelis–Menten isotherms fitted to the experimental data. Bars indicate standard errors ($N=5$)

(Fig. 4). There were no significant differences in mineralization rates of glucose, acetate, and citrate between the rhizosphere and bulk soil fractions under the Dipterocarp and Macaranga trees, respectively (Fig. 4).

The data of LMWOS mineralization rates were fitted well to the single Michaelis–Menten kinetic equation ($R^2>0.98$; Fig. 4, Table 4). Michaelis–Menten kinetic parameters (V_{max} and K_M) describe microbial capacity to mineralize substrate and microbial response to substrate availability, respectively. An increase in malate and oxalate mineralization activity in the Dipterocarp rhizosphere (Fig. 4) was caused by the higher V_{max} values, compared to the bulk soil (Table 4). An increase in malate mineralization activity in the Macaranga rhizosphere (Fig. 4) was caused by the lower K_M values, compared to the bulk soil (Table 4).

Carbon fluxes of low molecular weight organic substance mineralization in the rhizosphere

Using the bulk density, the volumes of the rhizosphere and bulk soil fractions, the mineralization kinetics (Table 4), we quantified soil C fluxes of LMWOS mineralization to test whether root exudation and rhizosphere C fluxes are quantitatively important relative to the bulk soil C cycles. Monosaccharides, malate, and citrate were major substrates for microbial LMWOS mineralization in the bulk soil, while malate accounted for the majority of mineralization C fluxes by in the rhizosphere (Fig. 5). When mean residence times (MRTs) of LMWOAs and monosaccharides were calculated by dividing the amount of LMWOS-C in soil solution by mineralization C

Table 4 Michaelis–Menten kinetic parameter values of the rhizosphere and bulk soil fractions

Site	Fraction	Glucose		Acetate		Oxalate		Malate		Citrate	
		V_{max} (nmol g^{-1})	K_M (μM)	V_{max} (nmol g^{-1})	K_M (μM)	V_{max} (nmol g^{-1})	K_M (μM)	V_{max} (nmol g^{-1})	K_M (μM)	V_{max} (nmol g^{-1})	K_M (μM)
Dipterocarp	Rhizosphere	292 ± 48 A	9446 ± 1912 A	73 ± 11 A	320 ± 41 B	123 ± 9 A	405 ± 42 A	170 ± 10 A	86 ± 11 A	42 ± 3 A	67 ± 11 B
	Bulk	303 ± 80 A	12,095 ± 3909 A	68 ± 13 A	1176 ± 262 A	78 ± 24 B	407 ± 12 A	170 ± 10 A	86 ± 11 A	42 ± 3 A	67 ± 11 B
Macaranga	Rhizosphere	353 ± 63 a	13,054 ± 2786 a	232 ± 86 a	3863 ± 1656 a	47 ± 15 a	424 ± 22 a	97 ± 7 a	61 ± 8 b	71 ± 9 a	288 ± 63 a
	Bulk	210 ± 48 a	9424 ± 2761 a	353 ± 131 a	6117 ± 2520 a	45 ± 10 a	441 ± 16 a	63 ± 5 b	85 ± 12 a	53 ± 11 a	278 ± 96 a

Mean ± standard errors ($N = 5$). Within each column of each site, different letters (A, B or a, b) indicate that values are significantly ($P < 0.05$) different between rhizosphere and bulk soil fractions

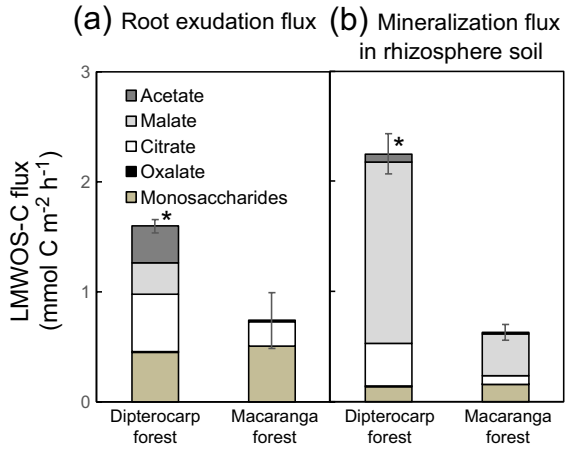

Fig. 5 **a** Basal area-weighted mean root exudation rates of low molecular weight organic substances (LMWOSs) and (**b**) C flux of LMWOS mineralization in the rhizosphere soil fraction. Bars indicate standard errors for the sum of LMWOSs ($N = 5$). Significant difference ($P < 0.05$) between sites was tested by ANOVA

flux, MRTs were short ranging from 0.2 h to 5.8 h for LMWOAs and 10.9 h to 15.0 h for monosaccharides, respectively (Table 5). Malate exhibited the shorter MRTs among LMWOSs (Table 5). When the mineralization C fluxes per soil mass in the rhizosphere were compared to the bulk soil, the rhizosphere effects differed between tree species and between LMWOSs (Table 5). The higher rhizosphere effects were observed for acetate, oxalate, and malate under the Dipterocarp trees, compared to the Macaranga trees (Table 5).

Discussion

Effects of tree species on root exudation rates

Judging from the fact that LMWOSs are rapidly consumed by microbial uptake and sorption, LMWOS dynamics could be strongly regulated by soil types, substrate charges, and root and microbial activities (Van Hees et al. 2003). On the other hand, LMWOS-C flow in the rhizosphere soil is dependent primarily on composition and amounts of root exudation (Grayston et al. 1996; Aoki et al. 2012). The higher concentration of malate and oxalate in the Dipterocarp rhizosphere relative to the bulk soil suggests that the pool size of organic acids could also vary between

Table 5 Soil solution pool, mineralization flux, and mean residence time of organic acids in the rhizosphere and bulk fractions of soils

Site		Soil solution pool					Mineralization C flux					Mean residence time				
Fraction	Mass ftac-tiona	Monosac-charides	Acetate	Oxalate	Malate	Citrate	Monosac-charides	Acetate	Oxalate	Malate	Citrate	Mono-saccha-rides	Ace-tate	Oxa-late	Malate	Citrate
	(%)	(mmol C m^{-2})					(mmol C m^{-2} h^{-1})					(h)				
Dipterocarp forest																
Rhizos-phere	5.5	1.52 ± 0.22	0.11 ± 0.02	0.007 ± 0.002	0.39 ± 0.04	0.25 ± 0.06	0.14 ± 0.03	0.07 ± 0.01	0.007 ± 0.001	1.65 ± 0.17	0.38 ± 0.05	10.9 a	1.5b	l.lb	0.2 a	0.7 b
Bulk	94.5	19.92 ± 3.52	1.41 ± 0.39	0.018 ± 0.001	2.01 ± 0.88	5.68 ± 1.32	1.49 ± 0.48	0.24 ± 0.05	0.010 ± 0.000	6.13 ± 0.61	5.18 ± 0.62	13.4a	5.8 a	1.7 a	0.3 a	1.1 a
Rhizos-phere effect							**1.6 ± 0.6**	**5.1** ± 1.3	**11.3** ± 1.2 a	**4.6** ± 0.7 a	1.3 ± 0.2					
Macaranga forest																
Rhizos-phere	8.3	1.93 ± 0.36	0.07 ± 0.03	0.007 ± 0.003	0.09 ± 0.02	0.11 ± 0.03	0.16 ± 0.03	0.01 ± 0.01	0.002 ± 0.000	0.38 ± 0.06	0.08 ± 0.02	12.4 A	5.6 A	3.0 A	0.2 B	1.4 B
Bulk	91.7	16.49 ± 3.13	2.44 ± 0.38	0.018 ± 0.005	1.30 ± 0.25	1.89 ± 0.37	1.10 ± 0.32	0.42 ± 0.17	0.005 ± 0.000	2.70 ± 0.44	1.06 ± 0.36	15.0 A	5.8 A	3.3 A	0.5 A	1.8A
Rhizos-phere effect							**1.6 ± 0.6 a**	0.3 ± 0.2 b	**4.6** ± 0.3 b	**1.6 ± 0.4** b	0.8 ± 0.3					

[a]The percentage of the rhizosphere and bulk soil fractions relative to the total soil mass

[b]MRT (mean residence time) of organic acids was calculated by dividing soil solution pool by mineralization flux

Mean ± standard errors ($N = 5$)

Within each column of each site, different letters (A, B or a, b) indicate that values are significantly ($P < 0.05$) different between rhizosphere and bulk soil fractions or between sites

tree species, depending on the supply of organic acids (Fig. 1). Consistent with the hypothesis, the composition and rates of root exudation differ between Dipterocarp and Macaranga trees (Table 2; Fig. 5a). The greater rates of organic acid exudation from Dipterocarp trees (Table 2; Fig. 2a) are consistent with the finding that ectomycorrhizal fungi promote mineral weathering by releasing LMWOAs from the hyphae (Jongmans et al. 1997). The lower soil pH under Dipterocarp trees also increases allocation of photosynthate to organic acid exudation (Fig. 2b). Soil pH is used as a proxy of P deficiency and Al toxicity in our study, because P solubility decreases and Al^{3+} solubility increases along with soil acidification (Jones, 1998; Fujii et al. 2018). For plants' survival on acidic soils, the high levels of divalent organic acids in the rhizosphere need to be maintained to solubilize recalcitrant P from Al or Fe oxides or to detoxify Al^{3+} (Van Hees et al. 2005). Consistent with this, both soil available P and foliar P of Dipterocarp (0.25 mg g^{-1}) is lower than soil available P and foliar P of Macaranga (0.69 mg g^{-1}) (Table 1; Fujii et al. 2021). The Al concentration in the bulk soil solution under Dipterocarp trees is higher than that under Macaranga trees (Fujii et al. 2021). Soil acidification might lead to a shift of vegetation towards tree species with capacity to release divalent organic acids from roots, as seen by succession from pioneer tree species to dipterocarp tree species in our study (Fig. 2b). Recently Mn concentrations in the plant leaf is postulated as a proxy for organic acid exudation activities (Pang et al. 2018; Lambers, 2020), because organic acids increase Mn solubility and root Mn uptake. However, the pattern is not clear in our study, although Dipterocarp fresh leaf litter displays slightly higher Mn concentration range (0.37–0.65 mg g^{-1}) than Macaranga leaf (0.34 mg g^{-1}) (Fujii et al. 2020b).

Rhizosphere effects on organic acid mineralization

The rhizosphere is the hotspot of LMWOS cycles (Kuzyakov and Razavi, 2019). This is supported by the higher concentrations and C fluxes of malate mineralization, compared to the bulk soil in our study (Figs. 1 and 6). This is primarily due to the substrate inputs via root exudation (Jones et al. 1996; Butler et al. 2003), but microbial potentials to mineralize exudates are also elevated (Fig. 4; Fujii et al. 2013). This is evidenced by the higher

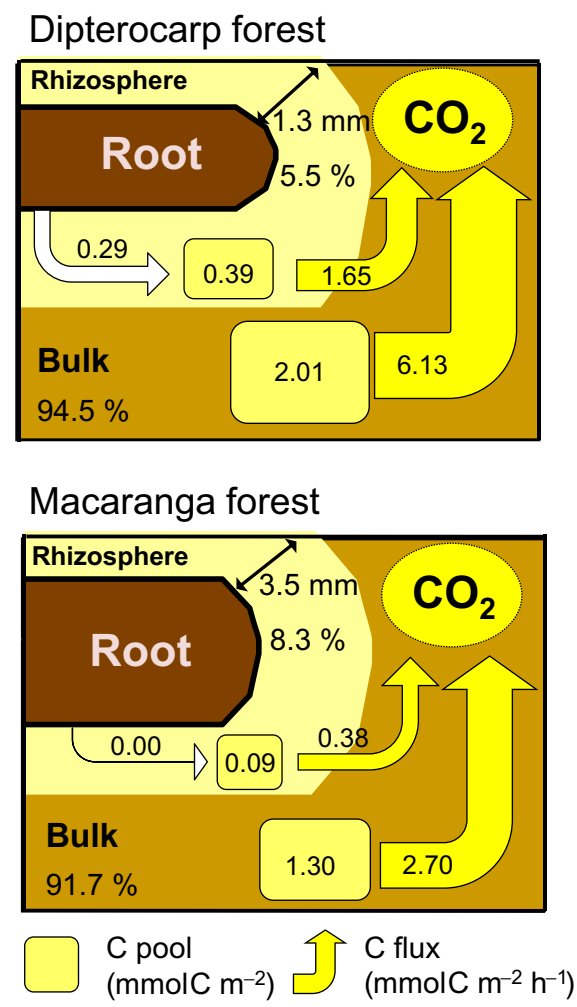

Fig. 6 Malate-C pools and fluxes of root exudation and mineralization in the rhizosphere and bulk soil fractions. The data of root exudation rates (Fig. 5), mineralization C flux (Table 5) were used

V_{max} values of malate mineralization in the Dipterocarp rhizosphere (Table 4). It has been shown that microbial mineralization potentials (transporter activity) could vary, depending on C sources that microbes have grown on (Jones et al. 1996). Microbial community grown on malate could have the higher activity of malate transporter that takes the malate into the cell, compared to microbial community grown without malate (Jones et al. 1996). In our study, root exudation of malate might increase malate preference and mineralization activity of rhizosphere microbial community (Table 4). This contrasts with the low availability of organic acids

in the volcanic soils leading to the low microbial mineralization activities (Fujii et al. 2019). Both higher substrate availability in the rhizosphere and the associated higher mineralization activities of microbes shape the hotspots of organic acids in the Dipterocarp rhizosphere (Fig. 5).

Compound-specific rhizosphere effects

Tropical trees require rhizosphere processes to acquire P and protect roots in the highly weathered and acidified soils (Fujii et al. 2018). Due to the highest sorption of malate and its shortest MRTs among organic acids (Fig. 3; Table 5), the efficacy of exuded malate on P mobilization and Al^{3+} detoxification in the rhizosphere could be reduced (Jones et al. 1996). There should occur the mechanisms to maintain the pool size of malate in rhizosphere soil solution (Van Hees et al. 2005). This could be partly accounted for by the higher rates of malate exudation from the Dipterocarp roots (Table 2), but malate-C inputs by root exudation can not account for the whole C fluxes of malate mineralization in the rhizosphere (Figs. 5 and 6). This indicates that malate could be supplied by the other sources as well as root exudation. Malate could be produced as microbial metabolites in organic matter degradation pathways such as tricarboxylic acid cycle (Van Hees et al. 2005). Degradation of the native organic matters or root litters is also the malate source, because positive priming effects can be induced by root exudates in the rhizosphere, where the exuded organic acids could destabilize organic-mineral associations or aggregates (Keiluweit et al. 2015; Ding et al. 2021). Malate as well as oxalate can also be released by fungi in lignin oxidation by manganese peroxidase, where malate chelates Mn^{3+} and complex of Mn^{3+}-malate works as diffusible oxidant (Hatakka, 2001). The exuded glucose or citrate might be transformed into malate and released by fungi to decompose lignin or recalcitrant organic matter (Plassard and Fransson, 2009). Organic acid exudation from roots increases at lower pH for P solubilization and Al^{3+} detoxification (Fig. 2b) and for degradation of lignin-rich dipterocarp litters (Fujii et al. 2020b). These rhizosphere microbial activities, as well as root exudation, could increase malate turnover and affect C fluxes at soil profile and ecosystem scales.

Conclusions

Root exudation rates differ between tree species. Malate was a dominant organic acid exuded from Dipterocarp roots, while monosaccharides were dominant exudates of Macaranga trees. Organic acid exudation increased with increasing root surface area and with decreasing soil pH. Root exudation of malate increases malate mineralization activities by rhizosphere microbes, but malate budgets suggest that malate rhizosphere microbes are another malate producer. Tree species affects both root exudation composition and rhizosphere microbes that increase malate production at lower soil pH, likely for phosphorus solubilization, aluminum detoxification, and lignin degradation.

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Author contributions K.F. and C.H. designed the study. K.F. and S. established the field experiment and discussed the results. K.F. wrote the manuscript.

Data availability The data of soil solution composition, organic acid sorption, and mineralization kinetics are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.2z34tmpmg (K. Fujii).

Declarations

Competing interests There are no completing interests.

References

Aoki M, Fujii K, Kitayama K (2012) Environmental control of root exudation of low-molecular-weight organic acids in tropical rainforests. Ecosystems 15:1194–1203

Blakemore LC, Searle PL, Daly BK (1987) Methods for chemical analysis of soils. NZ Soil Bureau Scientific Report, 80

Burney CM, Sieburth JMcN, (1977) Dissolved carbohydrates in seawater. II. A spectrophotometric procedure for total carbohydrate analysis and polysaccharide estimation. Mar Chem 5:15–28

Butler JL, Williams MA, Bottomley PJ, Myrold DD (2003) Microbial community dynamics associated with rhizosphere carbon flow. Appl Environ Microbiol 69:6793–6800

Dijkstra FA, Carrillo Y, Pendall E, Morgan JA (2013) Rhizosphere priming: a nutrient perspective. Front Microbiol 4:216

Ding W, Cong WF, Lambers H (2021) Plant phosphorus-acquisition and -use strategies affect soil carbon cycling. Trends Ecol Evol https://doi.org/10.1016/j.tree.2021.06.005

Fujii K, Aoki M, Kitayama K (2013) Biodegradation of low molecular weight organic acids in rhizosphere soils from a tropical montane rain forest. Soil Biol Biochem 47:142–148

Fujii K, Shibata M, Kitajima K, Ichie T, Kitayama K, Turner BL (2018) Plant–soil interactions maintain biodiversity and functions of tropical forest ecosystems. Ecol Res 33:149–160

Fujii K, Hayakawa C, Inagaki Y, Ono K (2019) Sorption reduces the biodegradation rates of multivalent organic acids in volcanic soils rich in short-range order minerals. Geoderma 333:188–199

Fujii K, Hayakawa C, Inagaki Y, Kosaki T (2020) Effects of land use change on turnover and storage of soil organic matter in a tropical forest. Plant Soil 446:425–439

Fuji K, Nakada Y, Umezawa K, Yoshida M, Shibata M, Hayakawa C, Inagaki Y, Kosaki T, Hangs R (2020) A comparison of lignin-degrading enzyme activities in forest floor layers across a global climatic gradient. Soil Ecol Lett 2:281–294

Fujii K, Toma T, Sukartiningsih (2021) Comparison of soil acidification rates under different land uses in Indonesia. Plant and Soil, 1–17

Gamo M (2003) Measurements of net ecosystem production by eddy correlation method. The Tropical Forestry 57:7–16 ((in Japanese))

Giesler R, Lundström US (1993) Soil solution chemistry-the effects of bulking soil samples and spatial variation. Soil Sci Soc Am J 57:1283–1288

Grayston SJ, Vaughan D, Jones D (1996) Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and impact on microbial activity and nutrient availability. Appl Soil Ecol 5:29–56

Hatakka A (2001) Biodegradation of lignin. In: Hofrichter M, Steinbüchel A (eds) Biopolymers: biology, chemistry, biotechnology, applications, vol 1. Lignin, humic substances and coal. Wiley VCH, Weinheim, pp 129–180

Johnson KM, Sieburth JMcN, (1977) Dissolved carbohydrates in seawater. I. A precise spectrophotometric analysis for monosaccharides. Mar Chem 5:1–14

Jones DL (1998) Organic acids in the rhizosphere -a critical review. Plant Soil 205:25–44

Jones DL, Brassington DS (1998) Sorption of organic acids in acid soils and its implications in the rhizosphere. Eur J Soil Sci 49:447–455

Jones DL, Prabowo AM, Kochian LV (1996) Kinetics of malate transport and decomposition in acid soils and isolated bacterial populations: the effect of microorganisms on root exudation of malate under Al stress. Plant Soil 182:239–247

Jones DL, Dennis PG, Owen AG, Van Hees PAW (2003) Organic acid behavior in soils –misconceptions and knowledge gaps. Plant Soil 248:31–41

Jones DL, Hodge A, Kuzyakov Y (2004) Plant and mycorrhizal regulation of rhizodeposition. New Phytol 163:459–480

Jongmans AG, Van Breemen N, Lundström U, Van Hees PAW, Finlay RD, Srinivasan M, Olsson M (1997) Rock-eating fungi. Nature 389(6652):682

Keiluweit M, Bougoure JJ, Nico PS, Pett-Ridge J, Weber PK, Kleber M (2015) Mineral protection of soil carbon counteracted by root exudates. Nat Clim Chang 5:588–595

Kuzyakov Y, Razavi BS (2019) Rhizosphere size and shape: Temporal dynamics and spatial stationarity. Soil Biol Biochem 135:343–360

Lambers, H. et al. (2020) Leaf manganese concentrations as a tool to assess belowground plant functioning in phosphorus-impoverished environments. Plant and Soil, 1-19

Ma Z, Guo D, Xu X, Lu M, Bardgett RD, Eissenstat DM, Hedin LO (2018) Evolutionary history resolves global organization of root functional traits. Nature 555(7694):94

Pang J, Ruchi B, Zhao H, Bansal R, Bohuon E, Lambers H, Ryan MH, Ranathunge K, Siddique KMH (2018) The carboxylate-releasing phosphorus-mobilising strategy could be proxied by foliar manganese concentration in a large set of chickpea germplasm under low phosphorus supply. New Phytology 219:518–529

Phillips RP, Erlitz Y, Bier R, Bernhardt ES (2008) New approach for capturing soluble root exudates in forest soils. Funct Ecol 22:990–999

Plassard C, Fransson P (2009) Regulation of low-molecular weight organic acid production in fungi. Fungal Biol Rev 23:30–39

Slik JF, Keßler PJ, van Welzen PC (2003) Macaranga and Mallotus species (Euphorbiaceae) as indicators for disturbance in the mixed lowland dipterocarp forest of East Kalimantan (Indonesia). Ecol Indic 2:311–324

Smits WT (1994) Dipterocarpaceae: mycorrhizae and regeneration. Wageningen University and Research

Strobel BW (2001) Influence of vegetation on low-molecular-weight carboxylic acids in soil solution-a review. Geoderma 99:169–198

Ström L, Owen AG, Godbold DL, Jones DL (2001) Organic acid behavior in a calcareous soil: sorption reactions and biodegradation rates. Soil Biol Biochem 33:2125–2133

Toma T, Matius P, Kiyono Y, Watanabe R, Okimori Y (2000) Dynamics of burned lowland dipterocarp forest stands in Bukit Soeharto, East Kalimantan. In Rainforest Ecosystems of East Kalimantan (pp. 107–119). Springer, Tokyo

Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. Soil Biol Biochem 19:703–707

Van Hees PAW, Dahlen J, Lundström US, Boren H, Allard B (1999) Determination of low molecular weight organic acids in soil solution by HPLC. Talanta 48:173–179

Van Hees PAW, Vinogradoff SI, Edwards AC, Godbold DL, Jones DL (2003) Low molecular weight organic acid adsorption in forest soils: effects on soil solution concentrations and biodegradation rates. Soil Biol Biochem 35:1015–1026

Van Hees PAW, Jones DL, Finlay R, Godbold DL, Lundström US (2005) The carbon we do not see-the impact of low molecular weight compounds on carbon dynamics and respiration in forest soils: a review. Soil Biol Biochem 37:1–13

Walker TW, Syers JK (1976) The fate of phosphorus during pedogenesis. Geoderma 15:1–19

Wardle DA, Walker LR, Bardgett RD (2004) Ecosystem properties and forest decline in contrasting long-term chronosequences. Science 305:509–512

Wu J, Joergensen RG, Pommerening B, Chaussod R, Brookes PC (1990) Measurement of soil microbial biomass C by fumigation extraction: An automated procedure. Soil Biol Biochem 22:1167–1169

Zar JH (1999) Biostatistical analysis, 4th edn. Prentice-Hall, New Jersey