(Title)

In-situ measurement of the effect of canopy tree fine roots on nitrogen availability in forest soil

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(Declaration of interest)

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Abstract

Plant fine roots affect the amount of soil inorganic nitrogen (N) via their N uptake and their effects on organic matter decomposition and N mineralization. However, the effects of the fine roots of canopy trees on N availability in forest soil remain largely unknown owing to the limitations of *in-situ* measurements. Therefore, to reveal the effects of the fine roots of canopy trees on forest soil N availability, we developed a novel *in-situ* method that combines a modified *in-situ* resin-core method, using live fine roots, with a tree N demand estimation, using the N-balance method. Furthermore, we measured soil extracellular enzyme activities in the rhizosphere and bulk soils to determine whether fine roots stimulate soil enzyme activities. The results showed that soil enzyme activities were stimulated by the fine roots of canopy trees, especially in the rhizosphere, and this indicates that fine roots stimulate organic matter decomposition and N mineralization. However, fine roots had no significant effect on either the pool size or leaching of inorganic N. The estimated amount of potential N uptake by fine roots was greater than the amount of inorganic N released via fine root-induced N mineralization. Overall, our results indicate that almost all of the fine root-induced increment of mineralized inorganic N is taken up by the fine roots; thus, the fine roots of canopy trees did not affect N availability in forest soil. This study was limited by the fact that the fine roots in the resin cores were less dense than natural roots, and the fact that the actual root N uptake can differ from the estimated N uptake; these limitations should be resolved in future studies. Nevertheless, the proposed

method can be used to investigate the effects of fine roots on the N cycle in forest soil under field conditions considering N uptake and priming.

Keywords: Nitrogen cycle, Tree nitrogen uptake, Rhizosphere priming, Extracellular enzyme activities

Introduction

Nitrogen (N) availability in forest soils often limits the primary production of temperate forests (Vitousek and Howarth, 1991; LeBauer and Treseder, 2008). Most soil N is bound in complex macromolecular soil organic matter (SOM), a form of N that plants cannot directly utilize in forest ecosystems (Bormann et al., 1977). Complex SOM is degraded into labile SOM through the activities of various enzymes; labile SOM is further decomposed by enzymes and finally mineralized into inorganic N (Geisseler et al., 2010). Because net N mineralization is significantly and positively correlated with the annual net primary production of forests (Reich et al., 1997), understanding N mineralization processes is key for understanding forest productivity.

Plant roots not only affect soil N pools through their uptake of soil N but also by altering the activities of enzymes involved in SOM decomposition via rhizodeposition (Brzostek et al., 2013; Yin et al., 2014; Meier et al., 2017), and this consequently affects the N mineralization rate in the rhizosphere (i.e., the soil surrounding plant roots). This rhizodeposition-induced alteration of the decomposition and mineralization processes is called the rhizosphere priming effect (RPE) and has been measured mainly through the changes in soil respiration (Dijkstra et al., 2013). The RPE ranges from -50% to 380% and is influenced by factors such as plant type, plant growth stage, and environmental conditions, including soil temperature and nutrient conditions (Cheng et al., 2003,

2014; Cheng and Kuzyakov, 2005; Dijkstra and Cheng, 2007; Bengtson et al., 2012). Tree species tend to have a higher level of RPE than grassland or crop species (Yin et al., 2014; Huo et al., 2017). Furthermore, most trees in temperate forests have symbiotic relationships with mycorrhizal fungi (Marschner and Dell, 1994; Deckmyn et al., 2014). Besides helping their host plants to take up nutrients, including N, mycorrhizal fungi deliver photosynthetically produced organic carbon (C) from the host to other parts of the fungus via their hyphae, which promotes the degradation of organic N that is far from the rhizosphere (Marschner and Dell, 1994; van Hees et al., 2006; Toljander et al., 2007; Talbot and Treseder, 2010). Therefore, mycorrhizal symbiosis would extend the priming effects of plant roots into not only the rhizosphere but also the bulk soil. However, most of these findings are based on studies of the priming effect on C dynamics, rather than N dynamics. Although studies using herbaceous species and seedlings and saplings of woody species have shown that the priming effect also affects soil N availability (Dijkstra et al., 2009; Bengtson et al., 2012; Cheng et al., 2014), there is relatively limited information on the priming effects of the fine roots of canopy trees on forest soil N dynamics owing to methodological limitations.

Specifically, nitrogen mineralization processes, including ammonification and nitrification, in forest soils have been investigated mainly using laboratory incubation methods and *in-situ* incubation methods (Kandeler, 1996). For *in-situ* incubations, the buried bag and resin core methods have been widely used (e.g. Hart and Firestone 1989; Zou et al. 1992; Kolberg et al. 1997; Shibata et

al. 2011; Urakawa et al. 2014). Studies using these methods have revealed that N transformation is affected by environmental factors, such as temperature, moisture content, and C and N concentrations (Kladivko and Keeney, 1987; Tietema et al., 1992; Yan et al., 2008; Shibata et al., 2011). However, as these traditional methods exclude the effects of live plant roots, they may overestimate or underestimate N transformation processes (Frank and Groffman, 2009). Furthermore, studies on the priming effects of the fine roots of canopy trees on the N cycle have predominantly compared the N mineralization potential of field-sampled rhizosphere soil to that of bulk soil using short-term laboratory incubations without live roots (Brzostek et al., 2013; Yin et al., 2014; Lin et al., 2018). Comparisons made using this technique reveal the potential RPE on N mineralization; however, the method still ignores the organic substrate supply and nutrient uptake by fine roots and mycorrhiza during incubation. Nitrogen diffusion according to concentration gradients and N loss through leaching are also not considered in the laboratory incubation method. Therefore, *in-situ* measurements with live roots are necessary to understand the potential effects of the fine roots of canopy trees on the N cycle in forest soils under natural conditions.

Here, we aim to reveal how canopy tree roots affect forest soil N dynamics via uptake and priming. For this purpose, we developed a new method that combines *in-situ* incubation and root N uptake estimation. For *in-situ* incubation, we modified the resin core method by inserting an intact root system of a canopy tree into the side of an unplasticized polyvinyl chloride (uPVC) pipe (Fig. 1).

78 Thereafter, the changes in the extractable N content in the incubated soil and N leaching from the
79 bottom of the pipe were measured; net inorganic N increment was defined as the total increase in
80 inorganic N pools and leaching. To estimate the potential N uptake by fine roots in the plots and pipes,
81 the above- and belowground net primary production were calculated based on litterfall, fine root
82 production, and the biomass growth estimated using allometric relationships. The amount of change
83 in the inorganic N pools change that was induced by the fine roots of canopy trees was evaluated by
84 comparing the net inorganic N increments and potential N uptakes between incubations conducted
85 with and without live roots. As the RPE and hyphosphere priming effect vary with nutrient availability
86 (Phillips and Fahey, 2008), we investigated the differences in the magnitude of the effects of fine roots
87 on soil N availability between soil with and without an organic horizon. The activities of four enzymes
88 were measured at the end of the incubation to assess the positive effects that rhizosphere and
89 hyphosphere priming had on N mineralization through their stimulation of SOM decomposition.
90 Studies have reported that tree roots significantly accelerate nutrient cycling, and that this acceleration
91 is quantitatively important (Finzi et al., 2015; Huo et al., 2017). At the same time, clear-cutting of trees
92 increases ecosystem N losses (Vitousek et al., 1979), indicating that trees increase N retention in the
93 forest ecosystem through their uptake and storage of N in their biomass. Therefore, we hypothesize
94 that (1) the fine roots of canopy trees will stimulate enzyme activities, resulting in the stimulation of
95 N mineralization via enhanced SOM decomposition. However, (2) the amount of N taken up by fine

roots will exceed the amount of N mineralized as a result of priming effects. Therefore, (3) fine roots will reduce the size of the inorganic N pools within the pipes and the extent of N leaching from the bottom of the pipes, indicating that canopy trees increase N retention in the forest ecosystem.

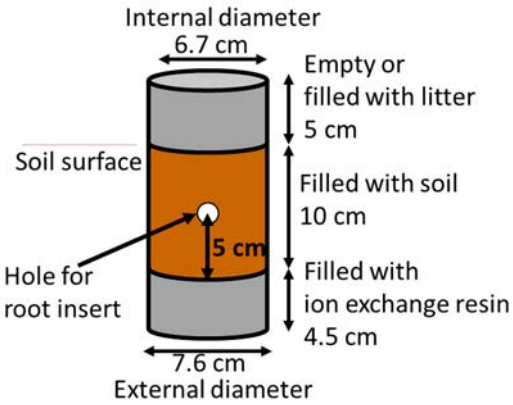


Fig. 1 Schematic of the incubation pipe used in this study.

Experimental

Study site

The study site was a deciduous broad-leaved temperate forest in the Shibecha Branch of the Hokkaido Forest Research Station at the Field Science Education and Research Center of Kyoto University (43°24.2′N, 144°38.5′E). The soil in this site has been characterized as an Andosol (IUSS Working Group WRB, 2015). According to data from a meteorological station located approximately 9 km south of the study site, the mean annual precipitation and air temperature are 1189 mm and 6.3 °C

(1986–2015), respectively. Soil freezing occurs from December to May, and the soil is covered by snow from November to April (Hosokawa et al., 2017).

We established four plots (15 m × 15 m) in the pure forest of *Quercus crispula* Blume. Each plot was separated from other plots by at least 30 m. The only canopy tree species in the study plots was *Q. crispula*, and the understory of the forest stand was densely covered with dwarf bamboo (*Sasa nipponica* Makino et Shibata). *Quercus crispula* has symbiotic relationships with ectomycorrhizal and arbuscular mycorrhizal fungi (Obase et al., 2007) and is a common tree species in the cool temperate forests of Japan (Hiura, 2001; Ohsawa et al., 2011). The number of *Q. crispula* in the study plots was 7–10, and its root density at the depth of 0–10 cm was 132.9 ± 47.4 g m^{-2} (Nakayama and Tateno, 2018). Most of the fine roots were distributed in the soil at depths of 0–50 cm (Gale and Grigal, 1987), and the vertical fine root productivity decreased with increasing soil depth, as did the fine root density (Fukuzawa et al., 2007). Therefore, almost all N for *Q. crispula* was taken up by the fine roots located within the top 50 cm of the soil. As 53.7% of the fine roots in the top 50 cm of the soil were concentrated at a depth of 0–10 cm in the adjacent plot (Tateno et al., 2020), the fine root density at the depth of 0–50 cm in the study plot was estimated as 247.4 ± 88.3 g m^{-2}. Details of study site and plots can be found in previous reports (Hosokawa et al., 2017; Nakayama and Tateno, 2018; Nakayama et al., 2019; Watanabe et al., 2019; Tateno et al., 2020).

In-situ incubation

Before incubation, the volumes of the mineral soil and the organic horizon used in the incubation were determined by sampling two surface soil samples (0–10 cm-depth) and one organic horizon from each plot on May 28, 2016; mineral soil was sampled using a soil core (surface area; 20 cm^2) and the organic horizon was manually collected from a 40 cm × 40 cm area. The soil samples were passed through a 4-mm mesh sieve to remove fine roots, gravel, and organic material. The organic horizon was divided into three groups (able to pass through a 9 mm mesh sieve, larger than 9 mm and partially decomposed, and larger than 9 mm and relatively fresh). After sieving and separating, the total weights of the mineral soil and organic horizon samples were measured. Thereafter, each sample was divided, and a subsample from each sample was oven-dried (at 70 °C for more than 72 h) to measure its moisture content. To calculate the water content of each wet soil and organic horizon sample, we multiplied the wet weight of the entire sample by the moisture content of its oven-dried subsample. Then, the bulk density and total dry weight of the organic horizon in the plots was calculated by subtracting the weight of the water contents from that of the total wet soil and organic horizon.

Soil samples (from the 0–10-cm depth) for the incubation were collected on June 7, 2016 from four randomly selected points in each plot and pooled to form one composite sample per plot. The soil samples were passed through a 4 mm mesh sieve and stored at 4 °C until further treatment. A

147 part of the newly collected soil was oven-dried at 70 °C for 72 h to measure the moisture content.
148 Based on the above-mentioned soil bulk density and moisture content of the newly collected soil, we
149 determined the soil (wet) weight to be used in the incubation. The bulk density of soil in the incubation
150 pipes was the same as that of the surrounding soil. We also determined the weights of the three groups
151 of sieved organic horizon material for incubation and used amounts that were consistent with the dry
152 weight of the plots on a per area basis. Some of the soil samples were used to measure the initial soil
153 extractable N concentration, as described below. The pH of the soil used in the incubation was 5.0 ±
154 0.1. The total C and N contents of the mineral soil and organic horizon used in the incubation are
155 described in Table 1.

156 The prepared soils were incubated *in-situ* using an altered resin-core method with uPVC
157 pipes (VP65; Sekisui chemical CO., LTD, Osaka, Japan; inner diameter: 6.7 cm, height: 15 cm; Fig.
158 1). We pierced a hole on the side of half of the pipes for the roots (diameter: 8 mm; the center of the
159 hole was 5 cm from the bottom of the pipe). Short uPVC pipes (height: 4.5 cm) were filled with 45 g
160 wet weight of a mixed bed ion-exchange resin (MB-1; ORGANO CORPORATION, Tokyo, Japan)
161 and covered with nylon mesh to retain the resin. The short pipes were placed at the bottom of all
162 incubation pipes to capture the inorganic N leached during the incubation period (Fig. 1). Soil was
163 prevented from escaping from the bottom of the incubation pipes by the nylon mesh. Sixty-four pipes
164 (32 pierced and 32 non-pierced) were used. On June 9, 2016, 32 live, intact fine-root systems of *Q.*

165 *crispula* (eight root systems from each plot) were excavated at a soil depth of 0–10 cm by hand.
166 Thereafter, 12.5 cm-deep holes were dug, taking care not to harm the excavated roots, and the pierced
167 pipes were inserted into the holes. The live intact root system (approximately 15 cm-long) was
168 carefully inserted into each pierced pipe through the hole in the pipes. The holes for root insertion
169 were then sealed with silicone sealant. For the 32 non-pierced pipes, we dug 12.5 cm-deep holes and
170 inserted the pipes into the holes without the live root systems. Soil was added and pushed gently from
171 the top of the pipes to fill 0–10 cm of the pipes and ensure that the bulk density of soil for incubation
172 was the same as that of the surrounding soils. Subsequently, we placed the sampled organic horizon
173 on top of the soils in half of the pipes with root systems and half of the pipes without root systems,
174 ensuring the same density and composition as the surrounding organic layer. The tops of the pipes
175 were covered with a rough polyester drainage net to prevent litter input during incubation, while still
176 allowing water flow from the upper surfaces of the pipes. Thus, four treatments were analyzed in this
177 study: with root system and organic horizon (RL), with root system but without organic horizon (R),
178 without root system but with organic horizon (CL), without root system or organic horizon (C). Four
179 replicates for each plot and treatment were incubated, with a total of 16 pipes for each plot and
180 treatment.

181 All incubation pipes were collected on June 5, 2017 (after an incubation period of 361 d).
182 We defined the soil adhering to the roots after gentle shaking as rhizosphere soil (Phillips and Fahey,

2006, 2008). Samples of bulk and rhizosphere soils were mixed for each treatment and plot. The roots incubated in two RL samples in plot 2 and one RL sample and two R samples in plot 3 were assumed to have died during the incubation based on their color and morphology (black and soft). Therefore, we did not mix them into the composite samples. After mixing, some of the bulk soil samples were oven-dried (70 °C, 72 h) to measure the moisture content. Because the amount of rhizosphere samples was limited, we assumed that the moisture content of rhizosphere samples was the same as that of bulk soils. The roots from each pipe were washed, oven-dried (70 °C, 72 h), and weighed.

Measurements of soil N concentrations

Pre-incubation wet soils and wet bulk soil samples were used to determine NO_3^--N (nitrate), NH_4^+-N (ammonium), and dissolved organic N (DON) concentrations after extraction with 0.5 M K_2SO_4 (extracted from 5 g of wet sample into 50 mL of K_2SO_4). Ion-exchange resin (10 g, wet weight) placed at the bottom of the incubation pipes was extracted twice with 50 mL of 1 M KCl. Ammonium and nitrate concentrations were measured by colorimetry using a microplate reader (Synergy HXT; BioTek, Winooski, VT, USA) with the indophenol blue method and Griess assay (Miranda et al., 2001). To measure the total extractable N (TEN), 1 mL extracts were autoclaved (121 °C, 20 min) with 1 mL of ion-exchanged water and 200 μL of alkalinity potassium persulfate solution. Thereafter, TEN was

measured using the same method used for nitrate measurements. The DON concentration of each sample was calculated by subtracting the summed of inorganic N (i.e., nitrate + ammonium) concentration from the TEN concentration. To measure the microbial biomass N (MBN) concentration, 10 g of wet soil was fumigated with chloroform. After 24 h of fumigation, the TEN content of fumigated soil was measured in the same manner described above. The MBN concentration was multiplied by 2.2 after subtracting the TEN concentration of the non-fumigated extracts from that of the fumigated extracts (Inubushi et al., 1984).

Measurement of soil extracellular enzyme activities

The enzyme assays were conducted within 24 h of sampling. The activities of four extracellular enzymes (Table S1) were measured using wet bulk and rhizosphere soil samples following the method of Saiya-Cork et al. (2002). Briefly, wet soil samples (2.0 g for bulk soil, 1.0 g for rhizosphere soil) were suspended in 200 mL (bulk soil) or 100 mL (rhizosphere soil) of acetate buffer (50 mM sodium acetate; pH 5.0). The soil suspensions were homogenized using a magnetic stirrer for 1 min. Subsequently, 200 μL aliquots of the suspensions were dispensed into 96-well microplates (eight replicate wells per sample per assay). Fifty microliters of a substrate (200 μM) was added into the sample wells. In the blank well, 50 μL of acetate buffer and 200 μL of sample suspension were

dispensed. Fifty microliters of 10 μM 4-methylumbelliferone (MUB) was added into the quenching standard well with 200 μL of sample suspension. The reference standard well had 200 μL of acetate buffer and 50 μL of 10 μM 4-MUB. The negative control well had 200 μL of acetate buffer and 50 μL of the substrate. The microplates were incubated at 20 °C in the dark for up to 2 h, and then the fluorescence was measured using a microplate reader (Synergy HXT; BioTek) with excitation and emission wavelengths of 360 and 460 nm, respectively. The target enzymes, incubation times, and substrates are listed in Table S1.

Quantification of net primary production and tree N demand

To estimate the net primary production of *Q. crispula*, litterfall and fine root growth were measured, and woody biomass (trunk, branch, and coarse root) production was estimated using the diameter at breast height (DBH) and the allometric relationships. To collect litterfall, eight litter traps (two for each plot; opening area: 0.25 m^2) were placed in the study plots. Litterfalls were collected once a month from September until all leaves of *Q. crispula* had fallen (September 26, October 18, and November 10, 2016). In the laboratory, the collected litter was oven-dried (at 60 °C for more than 72 h). We separated the dried litter into five categories (leaves of *Q. crispula*, leaves of other plants, small branches, large branches, and others) by hand sorting, and then weighed the litter. Others in the litter

237 trap (hereafter; other litter) included the acorns, flowers, and bud scales of *Q. crispula*. Fine root
238 growth was measured using the ingrowth core method. On July 5, 2016, eight cylindrical ingrowth
239 cores (mesh size: 2 mm, inner diameter: 3 cm, height: 10 cm) were buried in each plot (total 32 cores).
240 The cores were collected on August 15, 2017 (incubation period: 406 days). The cores contained roots
241 of *Q. crispula* and *S. nipponica*, which were manually separated according to their morphological
242 traits including color and their associated mycorrhiza. Both types of root were washed with water,
243 oven-dried (70 °C, 72 h), and weighed. The annual fine root growth of *Q. crispula* (0–50 cm depth)
244 was estimated from the fine root dry weight of *Q. crispula* in the ingrowth cores (0–10 cm) and vertical
245 fine root distribution (0–50 cm) in the adjacent plot, assuming that fine root growth is proportional to
246 fine root biomass. In other words, the production of fine roots of *Q. crispula*, as observed in ingrowth
247 cores, was divided by the incubation period and the proportion of fine root of *Q. crispula* in the 0–10-
248 cm soil layer of the adjacent plot (53.7%). To estimate woody biomass production, we measured the
249 DBH of each tree in the plot on July 13, 2016 and June 8, 2020. Two individuals died during 2016–
250 2020; therefore, the growth of these trees was assumed as zero. The growth of some individuals that
251 exhibited negative growth of DBH due to bark peeling was also assumed as zero. We used the
252 following allometric relationships for *Q. crispula* (Takagi et al., 2010):

253 $$Y_t = e^{(2.365 \times \log_e DBH - 2.596)}$$

254 $$Y_b = e^{(2.713 \times \log_e DBH - 4.456)}$$

255 $$Y_c = e^{(2.224 \times \log_e DBH - 2.918)}$$

256 where, Y_t, Y_b, and Y_c represent the biomass of the trunk, branch, and coarse root (kg), respectively.
257 These values were divided by four, which was the interval of DBH measurement (years), to estimate
258 the annual production. The annual net primary production (g m^{-2} $year^{-1}$) was estimated as the sum of
259 leaf litter, other litter, the estimated annual fine root growth (0–50 cm), and the estimated production
260 of the trunks, branches, and coarse roots of *Q. crispula*.

261 To estimate the annual N demand of *Q. crispula* (N_{demand}), we calculated its total N content
262 as the sum of the N contents of its leaf litter, other litter, fine roots, and woody parts (Aber et al., 1985;
263 Finzi et al., 2007; Tateno and Takeda, 2010; Lü et al., 2014). Before measuring the total N
264 concentration, leaf litter collected using the litter traps was mixed for each trap and species (n = 8),
265 and the fine roots collected from the ingrowth cores were mixed for each plot and species (n = 4).
266 Because the amount of other litter was limited, we composited all of other litter samples into one
267 before next step. Leaf litter, other litter, and fine roots were ground before measuring their total N
268 concentrations. The total N concentrations of the leaf litter, other litter, and roots were measured using
269 an elemental analyzer (Sumigraph NC-900; Sumika Chemical Analysis Service, Ltd.). The total N
270 concentrations of the branches, trunks, and coarse roots of *Q. crispula* were estimated as 0.58%, 0.39%,
271 and 0.45%, respectively (Takagi et al., 2010). The N_{demand} (g-N m^{-2} $year^{-1}$) of *Q. crispula* was
272 calculated by multiplying the annual net primary production by the N concentration in each part of *Q.*

273 *crispula* (Table 2). The potential N uptake per gram of dry *Q. crispula* fine roots in the plots under
274 natural conditions (Nat N_{uptake}; mg-N g^{-1} dry root $year^{-1}$) was estimated by dividing N_{demand} by the
275 density of *Q. crispula* fine roots in the plots (0–50 cm depth) (Table 2).
276
277 Data calculation and statistical analysis
278
279 The annual inorganic N increment (N_{inc}; g-N m^{-2} $year^{-1}$) and annual nitrate increment (Nit_{inc}; g-N m^{-2}
280 $year^{-1}$) were calculated based on the ammonium and nitrate pools and leaching, as listed in Table 2.
281 Because N inputs from rain and leachates of the organic horizon entering from the tops of the pipes
282 were not excluded to make the conditions inside the pipes as natural as possible, these N inputs were
283 included in N_{inc} and Nit_{inc}. The root-induced annual inorganic N increment under the conditions in the
284 incubation pipes ($N_{induced}$; g-N m^{-2} $year^{-1}$) was also estimated (Table 2). According to some reports,
285 nitrogen uptake by plants varies with soil N availability (e.g., Liao et al., 2018), whereas other studies
286 have reported the N availability is not significantly related to N uptake (e.g., Tateno and Takeda, 2010;
287 Maeda et al., 2018). Therefore, the "actual" N uptake by fine roots could vary with the environmental
288 conditions. However, to estimate the amount of potential N uptake in incubation pipes, we assumed
289 that the potential N uptake per unit fine root dry mass (g) in the incubation pipes was the same as that
290 under natural conditions. The potential amount of N taken up by the fine roots of *Q. crispula* under

291 the conditions in the incubation pipe (N_{uptake}; g-N m^{-2} $year^{-1}$) was calculated by multiplying Nat N_{uptake}
292 by the fine root density in each incubation pipe (Table 2).
293 The difference in the root densities within the pipes at the end of the incubation between the
294 two root treatments (R vs. RL) was tested using a t-test. The changes in the N concentrations during
295 the incubation were tested using a one-way analysis of variance (ANOVA) with a random effect
296 (random effect: sampling plot). To evaluate the differences in the soil properties (moisture content and
297 extractable N concentrations) and the amounts of leached inorganic N among the treatments (C, CL,
298 R, and RL), two-way ANOVAs with random effects (random effect: sampling plot) were used. To
299 analyze the differences in the soil extracellular enzyme activities between the bulk and rhizosphere
300 soils and among the treatments, we used a two-way ANOVA with a random effect (random effect:
301 sampling plot). The RPEs on the activities of soil extracellular enzymes were calculated by dividing
302 the enzyme activities in rhizosphere by those in the bulk soil. Furthermore, to compare the magnitude
303 of the RPE on enzyme activity, a one-way ANOVA with a random effect (random effect: sampling
304 plot) was performed for each type of enzyme examined. All statistical analyses were conducted using
305 R software (ver. 3.5.0, R Core Team, 2018).
306
307 **Results**
308

309 Root density and soil moisture content at the end of incubation

310

311 The root densities of R and RL in the pipes at the end of incubation were 66.63 ± 17.44 mg $pipe^{-1}$ and

312 63.06 ± 11.15 mg $pipe^{-1}$, which were equivalent to 18.90 ± 4.95 g m^{-2} and 17.89 ± 3.88 g m^{-2} (0–10

313 cm-depth) for R and RL, respectively. There was no significant difference between the root densities

314 of R and RL (t-test, $p = 0.745$). The moisture contents of the soil were 52.2 ± 1.2%, 52.9 ± 1.7%, 51.0

315 ± 0.9%, and 51.7 ± 0.8% for C, CL, R, and RL, respectively. Although the variation in moisture content

316 was only few percent, the effects of roots and organic horizon on the moisture content were statistically

317 significant (two-way ANOVA with a random effect; $F = 18.9$, $p < 0.01$ for roots and $F = 6.1$, $p < 0.05$

318 for the organic horizon). The interaction effect of roots and the organic horizon on moisture content

319 was not significant (two-way ANOVA with a random effect; $F = 0.0$, $p = 0.985$).

320

321 Changes in N concentration and differences in N concentration and leaching during incubation among

322 the treatments

323

324 Regardless of the treatment, the soil nitrate concentration after incubation was significantly higher

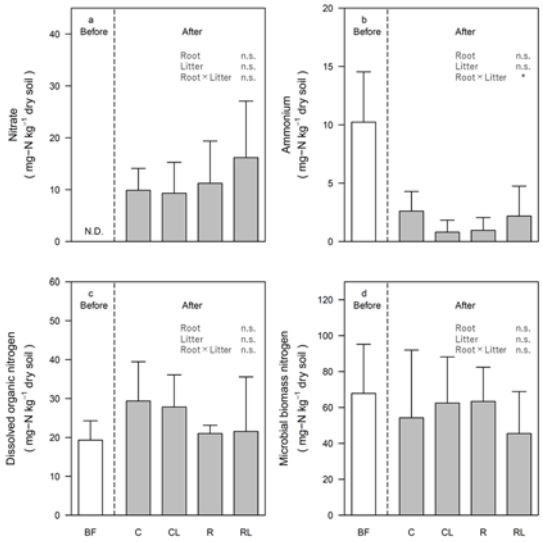
325 than that before incubation (one-way ANOVA with a random effect; $F = 12.7$, $p < 0.05$; Fig. 2a). The

326 roots and organic horizon had no significant effects on the concentration of nitrate after incubation

(Fig. 2a). In contrast, the soil ammonium concentration after incubation was significantly lower than that before incubation (one-way ANOVA with a random effect; $F = 75.1$, $p < 0.001$; Fig. 2b). A significant effect of the roots and organic horizon interaction on the ammonium concentration after incubation was detected using a two-way ANOVA with a random effect. We then divided the samples based on presence or absence of roots and organic horizons. Among samples without root systems (C and CL), the concentration of ammonium was significantly higher under C than under CL (one-way ANOVA with a random effect; $F = 18.0$, $p < 0.05$), whereas the organic horizon did not have a significant effect on the ammonium concentration in samples with root systems (one-way ANOVA with a random effect; R vs. RL, $F = 1.99$, $p = 0.254$). After dividing the samples into those with and without organic horizons, the roots did not have a significant effect on the ammonium concentration (one-way ANOVA with a random effect; $F = 2.23$, $p < 0.233$ for CL vs. RL and $F = 3.43$, $p = 0.161$ for C vs. R). The DON and MBN concentrations did not change significantly during incubation (one-way ANOVA with a random effect; $F = 1.30$, $p = 0.273$ and $F = 0.74$, $p = 0.404$, respectively). There was no significant difference in the DON and MBN concentrations after incubation among the treatments (Fig. 2c, d).

The amount of inorganic N leached from the bottom of the incubation pipes during the incubation ranged from 3.86 to 7.92 g-N m^{-2}; 96.1% ± 1.7% of the leached N was nitrate (Fig. 3). The amount of leached nitrate was significantly higher in samples with organic horizons (Fig. 3a). The

345 treatment type did not have a significant effect on the amount of leached ammonium (Fig. 3b).



346

347 **Fig. 2** Concentrations of (a) nitrate, (b) ammonium, (c) dissolved organic nitrogen, and (d) microbial

348 biomass nitrogen before (white bars) and after (gray bars) incubation. The bars and error bars represent

349 the mean and standard deviation, respectively (n = 4). N.D. indicates that the concentration was under

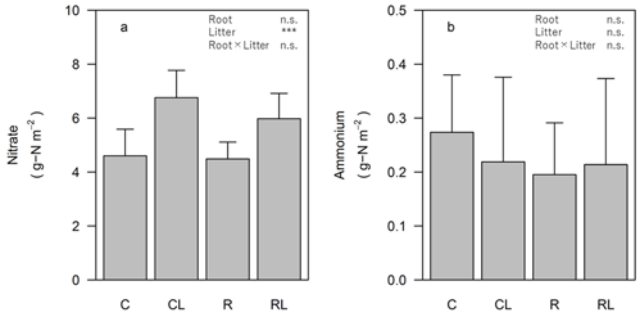
350 the detection limit. C, CL, R, and RL represent each treatment, with R indicating treatments with root

351 systems, C indicating those without root systems, and L indicating treatments with organic horizons

352 (i.e., litter). BF means before incubation and represents the initial soil. Root, Litter, and Root × Litter

353 in the upper right of each figure represent the results of the two-way ANOVA with a random effect.

354 The symbols are as follows: n.s.: $p \geq 0.05$, *: $p < 0.05$



355

356 **Fig. 3** Amounts of (a) nitrate and (b) ammonium leached from the bottom of the incubation pipes

357 during the incubation. The bars and error bars represent the mean and standard deviation, respectively

358 (n = 4). C, CL, R, and RL represent each treatment. Root, Litter, and Root × Litter in the upper right

359 of each figure represent the results of the two-way ANOVA with a random effect. The symbols are as

360 follows: n.s.: $p \geq 0.05$, ***: $p < 0.001$

361

362 Above- and belowground primary production of *Q. crispula*

363

364 The net primary production and N concentration in each part of *Q. crispula* are shown in Table 3. Leaf

365 production was the highest, followed by trunk, branch, and coarse-root production. The nitrogen

366 concentration was also the highest in the leaf litter (1.82% ± 0.08%, Table 3). Approximately three-

367 quarters of the N_{demand} of the canopy *Q. crispula* trees was from leaf litter production (Table 3).

368

369 Potential nitrogen uptake by *Q. crispula*, N budget, and root effect on the N budget

370

371 Based on the N_{demand} and fine root density of the study plots, the estimated Nat N_{uptake} was 28.58 ±

372 5.86 mg-N g^{-1} dry root $year^{-1}$. The N_{uptake} was estimated as 0.53 ± 0.15 g-N m^{-2} $year^{-1}$.

373 Regardless of treatment, positive Nit_{inc} and N_{inc} occurred (Table 4). Samples with organic

374 horizons presented higher Nit_{inc} and N_{inc} values than those without organic horizons (two-way ANOVA

375 with a random effect; $F = 119.5$, $p < 0.001$ and $F = 99.6$, $p < 0.001$, respectively), indicating N was

376 leached from the organic horizon. The effect of roots was not significant (two-way ANOVA with a

377 random effect; $F = 2.89$, $p = 0.123$ for Nit_{inc} and $F = 3.33$, $p = 0.101$ for N_{inc}, respectively), whereas

378 samples with root systems tended to have lower Nit_{inc} and N_{inc} values (Table 4). The effect of the root

379 and organic horizon interaction was not significant (two-way ANOVA with a random effect; $F = 1.79$,

380 $p = 0.214$ for Nit_{inc} and $F = 0.56$, $p = 0.473$ for N_{inc}, respectively).

381 The average $N_{induced}$ was positive, however, the magnitude of the effect differed among the

382 treatments (Table 4). The average $N_{induced}$ in samples with the organic horizon was almost zero and

383 lower than that in samples without the organic horizon (Table 4).

384

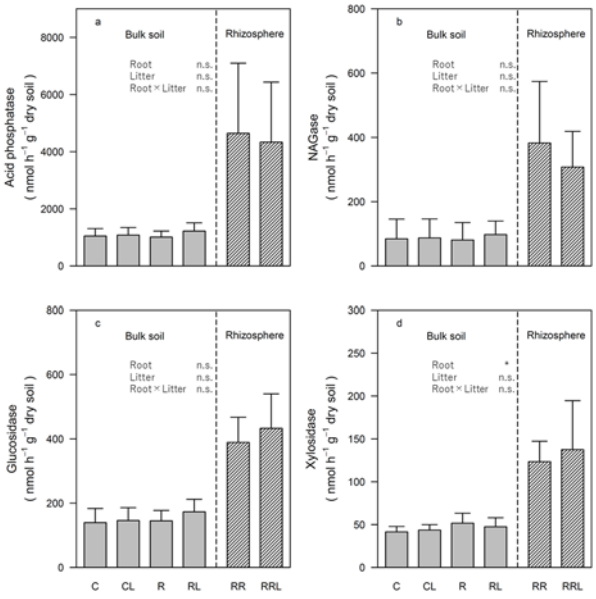
385 Enzyme activities in the rhizosphere and bulk soils

386

387 All enzyme activities investigated in this study were on average 2.7–4.4 times higher in the rhizosphere

388 soils than in the bulk soils, irrespective of the treatment (Fig. 4). The RPEs on the activities of acid

389 phosphatase and NAGase were slightly higher in samples without organic horizons (478% ± 286%
390 and 545% ± 313%, respectively) than in those with organic horizons (391% ± 241% and 338% ± 115%,
391 respectively); however, the difference was not statistically significant (one-way ANOVA with a
392 random effect; $F = 5.34$, $p = 0.104$ and $F = 1.54$, $p = 0.303$, respectively). The RPEs on the activities
393 of glucosidase and xylosidase were similar between samples with (253% ± 46% and 286% ± 71%,
394 respectively) and without (277% ± 70% and 244% ± 45%, respectively) organic horizons, and the
395 difference of treatments was not significant (one-way ANOVA with a random effect; $F = 0.58$, $p =$
396 0.502 and $F = 3.34$, $p = 0.165$, respectively). Within the treatments with organic horizons, all enzyme
397 activities in the bulk soils were slightly higher in the treatment with root systems than in that without
398 root systems (Fig. 4). This trend was not consistent for the activities of acid phosphatase and NAGase
399 in the treatments without organic horizons. In bulk soil with the organic horizon, the activities of
400 NAGase and glucosidase were slightly higher and the activity of xylosidase was lower than those in
401 bulk soil without the organic horizon (Fig. 4). However, the root system, organic horizon, and
402 interaction between the root system and organic horizon had no significant effects on the extracellular
403 enzyme activities in bulk soil, except for on xylosidase activity, which was significantly higher when
404 root systems were present (Fig. 4).



405

406 **Fig. 4** Activities of (a) acid phosphatase, (b) NAGase, (c) glucosidase, and (d) xylosidase in the bulk

407 soil (gray bars) and rhizosphere soil (shaded bars). The bars and error bars represent the means and

408 standard deviations, respectively (n = 4). C, CL, R, and RL represent each treatment. RR and RRL

409 represent the rhizospheres of R and RL, respectively. Root, Litter, and Root × Litter in the upper center

410 of each figure represents the results of the two-way ANOVAs with a random effect for the enzyme

411 activities in bulk soils. The symbols are as follows: n.s.: $p \geq 0.05$, ***: $p < 0.001$

412

413 **Discussion**

414

415 Plant roots directly affect soil N availability in two ways, that is, through N uptake and through their

416 influence on the decomposition and N mineralization processes via the priming effect. Although the

417 fine roots of *Q. crispula* did not significantly alter the N pools or N leaching in this study, this does

418 not suggest that the fine roots had no effect on N availability; rather, the fine roots accelerated these
419 processes and took up the excess N mineralized by this acceleration effect. Thus, in this study, we
420 showed that this is the mechanism by which the fine roots of canopy *Q. crispula* affect N availability
421 in forest soils by using our novel modified *in-situ* resin core method, which accounts for both N uptake
422 and priming by fine roots.

423 Our results did not contradict hypothesis (1), that is, extracellular enzyme activities were
424 stimulated by fine roots, and remarkably so in the rhizosphere (Fig. 4). This was consistent with the
425 findings of previous studies in both herbaceous and woody species (Phillips and Fahey, 2006; Zhu et
426 al., 2014; Kumar et al., 2016). Meier et al. (2017) reported that labile organic C inputs, including root
427 exudates, enhanced the rapid cycling of organic N and the rapid turnover of organic N-containing
428 compounds, such as chitin, which is the substrate for NAGase, and increased N availability in the soil.
429 The positive effect of roots on extracellular enzyme activities, especially xylosidase, was also
430 observed in bulk soil in this study, although NAG activity was slightly reduced by the presence of
431 fine roots in the absence of an organic horizon (Fig. 4). *Quercus crispula* has symbiotic relationships
432 with ectomycorrhizal fungi (Obase et al., 2007). Previous studies have shown that ectomycorrhizal
433 fungi extensively extend their mycelium into the bulk soil (Cairney, 2012; Soudzilovskaia et al., 2015),
434 expanding the effect of plant-derived C into soil that is distant from the roots (Meier et al., 2015;
435 Gorka et al., 2019). Xylo-oligomers, the substrate for xylosidase, are strong inhibitors of the

436 enzymatic degradation of recalcitrant organic matter (Qing et al., 2010). Thus, stimulation of
437 xylosidase activity would enhance recalcitrant organic matter decomposition. In other words, at least
438 a part of the root-distant soil area regarded as bulk (non-rhizosphere) soil in this study would be the
439 hyphosphere, and the fine roots of *Q. crispula* might accelerate the decomposition of recalcitrant
440 SOM in the bulk soil via the mycelium of their symbiotic mycorrhiza. Therefore, our results indicate
441 that the fine roots of *Q. crispula* stimulated the decomposition of recalcitrant SOM within the
442 rhizosphere and bulk soil, and enhanced the rates of N turnover and cycling, especially in the
443 rhizosphere. As an increase in SOM decomposition could increase N mineralization (Dijkstra et al.,
444 2009), the fine roots of *Q. crispula* might enhance N mineralization via rhizosphere and hyphosphere
445 priming effects. However, the excess inorganic N mineralized by the stimulatory effects of the roots
446 would then be readily taken up by the fine roots in the cores, as discussed below.

447 As for hypotheses (2) and (3), the presence of fine roots did not significantly affect either
448 the size of the N pool or the amount of inorganic N that was leached (Figs. 2 and 3), regardless of
449 their potential to stimulate SOM decomposition via the priming effect, suggesting that N_{uptake} could
450 be almost equal to $N_{induced}$ in this study (Table 4). In temperate forest soil, root-accelerated
451 mineralization can account for up to one-third of the total N mineralization (Finzi et al., 2015).
452 However, the magnitude and direction of the effect of plant roots on N availability and N loss by
453 leaching are determined by the balance between plant N uptake and priming. Dijkstra et al. (2009)

454 showed that the seedlings of woody species decreased the inorganic N pool by utilizing it along with
455 mineralized N; however, they observed a significant positive RPE on SOM decomposition. Herman
456 et al. (2006) reported that gross mineralization was approximately 10-times higher in rhizosphere soil
457 than in bulk soil, but N uptake by slender oats exceeded the amount of N supplied by N mineralization
458 in the rhizosphere. In this study, the lower density of fine roots in the incubation pipes suggested that
459 the amount of N taken up by the fine roots was lower than that under field conditions. The "actual"
460 uptake of N by fine roots also could differ from the "potential" estimated as N_{uptake} because N
461 availability might affect N uptake (Moreau et al., 2015; Liao et al., 2018). These are limitations of
462 this study. Therefore, further research is required to determine whether canopy trees increase N
463 retention in forest ecosystems. However, even if root-induced changes in N mineralization are
464 quantitatively important, our results suggest that the fine roots of canopy trees do not increase N loss
465 through leaching from the forest ecosystem because almost all of the N mineralized by root-
466 accelerated mineralization was immediately taken up by the trees.

467 Furthermore, when the organic horizon was present, $N_{induced}$ was almost zero (Table 4).
468 There are two possible explanations. One is that there was a lower RPE on N dynamics with higher
469 nutrient availability. The magnitudes of the RPEs on extracellular enzyme activities related to N and
470 phosphate acquisition in cores containing the organic horizon were slightly lower (Fig. 4). As
471 suggested by the differences in N leaching between C and CL (Fig. 3), some amount of substrates and

472 energy sources for microbes (including organic N) would be supplied by the organic horizon material
473 added to the cores; these supplements might lead to a reduced RPE. Based on the microbial nutrient
474 mining theory of RPE, root exudates are considered to stimulate SOM decomposition more in nutrient-
475 limited environments than in nutrient-rich ones (Craine et al., 2007; Fontaine et al., 2011; Dijkstra et
476 al., 2013; Cheng et al., 2014). For example, Phillips and Fahey (2008) reported that N fertilization
477 reduced the RPEs on microbial biomass, extracellular enzyme activity, and the net mineralization rate.
478 Moreover, although it is still unclear whether canopy trees increase root exudation under nutrient
479 limitation (e.g., Phillips et al. 2008, 2011; Meier et al. 2020), plants allocate more C to the
480 belowground parts under nutrient-limited conditions (Hill et al., 2007; Chowdhury et al., 2014). The
481 magnitude of the RPE is positively correlated with the quantity of labile C from fine roots (Yin et al.,
482 2013; Shahzad et al., 2015). Studies have suggested that a higher RPE results when nutrient
483 availability for plants is higher (Dijkstra et al., 2009; Bengtson et al., 2012); however, the release of
484 organic C is a significant C cost for plants (Uren, 2007). Although significant amounts of root exudates
485 have been observed under natural condition in this site (Nakayama and Tateno, 2018), it is still possible
486 that canopy trees decrease the priming effect on N mineralization by decreasing labile organic C
487 release when N limitation is less severe.

488 The second possible explanation for why $N_{induced}$ is almost zero in treatments with an organic
489 horizon is that the difference between “actual” and “potential” N uptake, which were derived from the

490 inappropriate assumption of our estimation. Moreau et al. (2015) investigated 11 crops and model
491 species and found that higher soil N availability led to higher N uptake per gram of fine roots, although
492 the extent of this effect varied by plant species. Maeda et al. (2018) reported that the combination of
493 soil N mineralization and belowground net primary production affected tree nitrogen uptake, while
494 soil N mineralization alone did not significantly correlate to tree N uptake in a natural deciduous-broad
495 leaved forest in East Hokkaido, the same region as that in this study. Indeed, the RPEs on the activities
496 of acid phosphatase and NAGase were slightly lower in the pipes with organic horizons than in those
497 without organic horizons, but the difference was not statistically significant. Therefore, there is a
498 possibility that the actual N uptake might be higher in the pipes with organic horizons than in those
499 without organic horizons because of nutrient availability, and the actual root-induced N increments
500 might be similar regardless of the difference in the treatment of the organic horizon.

501 In northern temperate forests, nitrogen is often regarded as the limiting factor for forest
502 primary production (Vitousek and Howarth, 1991; LeBauer and Treseder, 2008). At the same time, the
503 soil in the study site is an Andosol, which tend to have high total phosphorus (P) content, mainly as
504 organic P, but low available P (Borie and Rubio, 2003). Inorganic P deficiency increases the release of
505 phosphatase by plants and the activity of phosphatase (Almeida et al., 1999; Oberson et al., 2001).
506 This would explain why acid phosphatase activity was higher than the activities of the other enzymes
507 observed in this study. Therefore, our extracellular enzyme activity and N budget results might be

specific to Andosols. Further research using the modified resin-core method in other soil types will provide detailed insights into the relationships between canopy tree fine roots and N dynamics.

In this study, we modified the *in-situ* resin core method to investigate the effect of the fine roots of a specific tree species on the N cycle in forest soil. The results of this study suggest that the fine roots of *Q. crispula* stimulated SOM decomposition and N mineralization; however, they did not increase either the inorganic N pool size or N loss from the ecosystem because canopy trees took up almost all inorganic N mineralized by the priming effect of fine roots. Nevertheless, this study was limited by the fact that fine roots used in the cores were less dense than those under natural conditions, and the possibility that "actual" root N uptake differs from the "potential" estimated N_{uptake}. Furthermore, we only investigated the effect of roots of a single species and neglected the effects of interspecific interactions, whereas a natural forest ecosystem contains the roots of various plant species. However, the method used in this study is effective for investigating the relationships between canopy trees and forest soil N cycling, while considering both N uptake and priming. Future research using this method with higher root densities and with the fine roots of two or more tree species will provide more insights into the interactions between the fine roots of canopy trees and N cycling in forest soil.

References

Aber, J.D., Melillo, J.M., Nadelhoffer, K.J., McClaugherty, C.A., Pastor, J., 1985. Fine root turnover in forest ecosystems in relation to quantity and form of nitrogen availability: a comparison of two methods. Oecologia 66, 317–321. https://doi.org/10.1007/BF00378292

Almeida, J.P.F., Lüscher, A., Frehner, M., Oberson, A., Nösberger, J., 1999. Partitioning of P and the activity of root acid phosphatase in white clover (Trifolium repens L.) are modified by increased atmospheric CO2 and P fertilisation. Plant Soil 210, 159–166. https://doi.org/10.1023/A:1004625801141

Bengtson, P., Barker, J., Grayston, S.J., 2012. Evidence of a strong coupling between root exudation, C and N availability, and stimulated SOM decomposition caused by rhizosphere priming effects. Ecol. Evol. 2, 1843–1852. https://doi.org/10.1002/ece3.311

Borie, F., Rubio, R., 2003. Total and organic phosphorus in chilean volcanic soils. Gayana. Botánica 60, 69–78. https://doi.org/10.4067/S0717-66432003000100011

Bormann, F.H., Likens, G.E., Melillo, J.M., 1977. Nitrogen budget for an aggrading northern hardwood forest ecosystem. Science 196, 981–983. https://doi.org/10.1126/science.196.4293.981

Brzostek, E.R., Greco, A., Drake, J.E., Finzi, A.C., 2013. Root carbon inputs to the rhizosphere stimulate extracellular enzyme activity and increase nitrogen availability in temperate forest soils. Biogeochemistry 115, 65–76. https://doi.org/10.1007/s10533-012-9818-9

Cairney, J.W.G., 2012. Extramatrical mycelia of ectomycorrhizal fungi as moderators of carbon dynamics in forest soil. Soil Biol. Biochem. 47, 198-208. https://doi.org/10.1016/j.soilbio.2011.12.029

Cheng, W., Johnson, D.W., Fu, S., 2003. Rhizosphere effects on decomposition: Controls of plant species, phenology, and fertilization. Soil Sci. Soc. Am. J. 67, 1418–1427. https://doi.org/10.2136/sssaj2003.1418

547 Cheng, W., Kuzyakov, Y., 2005. Root effects on soil organic matter decomposition, in: Zobel, R.W.,
548 Wright, S.F. (Eds.), Roots and soil management: Interactions between roots and the soil. ASA-
549 CSSA-SSSA, Madison, WI, pp. 119–143. https://doi.org/10.2134/agronmonogr48.c7
550 Cheng, W., Parton, W.J., Gonzalez-Meler, M.A., Phillips, R., Asao, S., Mcnickle, G.G., Brzostek, E.,
551 Jastrow, J.D., 2014. Synthesis and modeling perspectives of rhizosphere priming. New Phytol. 201,
552 31–44. https://doi.org/10.1111/nph.12440
553 Chowdhury, S., Farrell, M., Bolan, N., 2014. Photoassimilated carbon allocation in a wheat plant-soil
554 system as affected by soil fertility and land-use history. Plant Soil 383, 173–189.
555 https://doi.org/10.1007/s11104-014-2173-y
556 Craine, J.M., Morrow, C., Fierer, N., 2007. Microbial nitrogen limitation increases decomposition.
557 Ecology 88, 2105–2113. https://doi.org/10.1890/06-1847.1
558 Deckmyn, G., Meyer, A., Smits, M.M., Ekblad, A., Grebenc, T., Kraigher, H., Komarov, A., 2014.
559 Simulating ectomycorrhizal fungi and their role in carbon and nitrogen cycling in forest
560 ecosystems. Can. J. For. Res. 44, 198–208. https://doi.org/10.1139/cjfr-2013-0496
561 Dijkstra, F.A., Bader, N.E., Johnson, D.W., Cheng, W., 2009. Does accelerated soil organic matter
562 decomposition in the presence of plants increase plant N availability? Soil Biol. Biochem. 41,
563 1080–1087. https://doi.org/10.1016/J.SOILBIO.2009.02.013
564 Dijkstra, F.A., Carrillo, Y., Pendall, E., Morgan, J.A., 2013. Rhizosphere priming: a nutrient perspective.
565 Front. Microbiol. 4, 216. https://doi.org/10.3389/fmicb.2013.00216
566 Dijkstra, F.A., Cheng, W., 2007. Interactions between soil and tree roots accelerate long-term soil carbon
567 decomposition. Ecol. Lett. 10, 1046–1053. https://doi.org/10.1111/j.1461-0248.2007.01095.x
568 Finzi, A.C., Abramoff, R.Z., Spiller, K.S., Brzostek, E.R., Darby, B.A., Kramer, M.A., Phillips, R.P.,
569 2015. Rhizosphere processes are quantitatively important components of terrestrial carbon and
570 nutrient cycles. Glob. Chang. Biol. 21, 2082–2094. https://doi.org/10.1111/gcb.12816

571 Finzi, A.C., Norby, R.J., Calfapietra, C., Gallet-Budynek, A., Gielen, B., Holmes, W.E., Hoosbeek, M.R.,
572 Iversen, C.M., Jackson, R.B., Kubiske, M.E., Ledford, J., Liberloo, M., Oren, R., Polle, A.,
573 Pritchard, S., Zak, D.R., Schlesinger, W.H., Ceulemans, R., 2007. Increases in nitrogen uptake
574 rather than nitrogen-use efficiency support higher rates of temperate forest productivity under
575 elevated CO2. Proc. Natl. Acad. Sci. 104, 14014–14019. https://doi.org/10.1073/pnas.0706518104
576 Fontaine, S., Henault, C., Aamor, A., Bdioui, N., Bloor, J.M.G., Maire, V., Mary, B., Revaillot, S.,
577 Maron, P.A., 2011. Fungi mediate long term sequestration of carbon and nitrogen in soil through
578 their priming effect. Soil Biol. Biochem. 43, 86–96.
579 https://doi.org/10.1016/J.SOILBIO.2010.09.017
580 Frank, D.A., Groffman, P.M., 2009. Plant rhizospheric N processes: what we don't know and why we
581 should care. Ecology 90, 1512–1519. https://doi.org/10.1890/08-0789.1
582 Fukuzawa, K., Shibata, H., Takagi, K., Satoh, F., Koike, T., Sasa, K., 2007. Vertical distribution and
583 seasonal pattern of fine-root dynamics in a cool-temperate forest in northern Japan: Implication of
584 the understory vegetation, Sasa dwarf bamboo. Ecol. Res. 22, 485–495.
585 https://doi.org/10.1007/s11284-006-0031-y
586 Gale, M.R., Grigal, D.F., 1987. Vertical root distributions of northern tree species in relation to
587 successional status. Can. J. For. Res. 17, 829–834. https://doi.org/10.1139/x87-131
588 Geisseler, D., Horwath, W.R., Joergensen, R.G., Ludwig, B., 2010. Pathways of nitrogen utilization by
589 soil microorganisms - A review. Soil Biol. Biochem. 42, 2058–2067.
590 https://doi.org/10.1016/j.soilbio.2010.08.021
591 Gorka, S., Dietrich, M., Mayerhofer, W., Gabriel, R., Wiesenbauer, J., Martin, V., Zheng, Q., Imai, B.,
592 Prommer, J., Weidinger, M., Schweiger, P., Eichorst, S.A., Wagner, M., Richter, A., Schintlmeister,
593 A., Woebken, D., Kaiser, C., 2019. Rapid transfer of plant photosynthates to soil bacteria via
594 ectomycorrhizal hyphae and its interaction with nitrogen availability. Front. Microbiol. 10, 168.

https://doi.org/10.3389/fmicb.2019.00168

Hart, S.C., Firestone, M.K., 1989. Evaluation of three in situ soil nitrogen availability assays. Can. J. For. Res. 19, 185–191. https://doi.org/10.1139/x89-026

Herman, D.J., Johnson, K.K., Jaeger, C.H., Schwartz, E., Firestone, M.K., 2006. Root influence on nitrogen mineralization and nitrification in Avena barbata rhizosphere soil. Soil Sci. Soc. Am. J. 70, 1504–1511. https://doi.org/10.2136/sssaj2005.0113

Hill, P.W., Marshall, C., Williams, G.G., Blum, H., Harmens, H., Jones, D.L., Farrar, J.F., 2007. The fate of photosynthetically-fixed carbon in Lolium perenne grassland as modified by elevated CO2 and sward management. New Phytol. 173, 766–777. https://doi.org/10.1111/j.1469-8137.2007.01966.x

Hiura, T., 2001. Stochasticity of species assemblage of canopy trees and understorey plants in a temperate secondary forest created by major disturbances. Ecol. Res. 16, 887–893. https://doi.org/10.1046/j.1440-1703.2001.00449.x

Hosokawa, N., Isobe, K., Urakawa, R., Tateno, R., Fukuzawa, K., Watanabe, T., Shibata, H., 2017. Soil freeze–thaw with root litter alters N transformations during the dormant season in soils under two temperate forests in northern Japan. Soil Biol. Biochem. 114, 270–278. https://doi.org/10.1016/j.soilbio.2017.07.025

Huo, C., Luo, Y., Cheng, W., 2017. Rhizosphere priming effect: A meta-analysis. Soil Biol. Biochem. 111, 78–84. https://doi.org/10.1016/j.soilbio.2017.04.003

Inubushi, K., Wada, H., Takai, Y., 1984. Determination of microbial biomass-nitrogen in submerged soil. Soil Sci. Plant Nutr. 30, 455–459. https://doi.org/10.1080/00380768.1984.10434710

IUSS Working Group WRB, 2015. International soil classification system for naming soils and creating legends for soil maps., World Reference Base for Soil Resources 2014, update 2015. https://doi.org/10.1017/S0014479706394902

Kandeler, K., 1996. Nitrogen Mineralization, in: Schinner, F., Öhlinger, R., Kandeler, E., Margesin, R.

(Eds.), Methods in Soil Biology. Springer Berlin Heidelberg, pp. 135–143. https://doi.org/10.1007/978-3-642-60966-4_9

Kladivko, E., Keeney, D., 1987. Soil nitrogen mineralization as affected by water and temperature interactions. Biol. Fertil. Soils 5, 248–252. https://doi.org/10.1007/BF00256909

Kolberg, R.L., Rouppet, B., Westfall, D.G., Peterson, G.A., 1997. Evaluation of an In Situ Net Soil Nitrogen Mineralization Method in Dryland Agroecosystems. Soil Sci. Soc. Am. J. 61, 504–508. https://doi.org/10.2136/sssaj1997.03615995006100020019x

Kumar, A., Kuzyakov, Y., Pausch, J., 2016. Maize rhizosphere priming: field estimates using 13C natural abundance. Plant Soil 409, 87–97. https://doi.org/10.1007/s11104-016-2958-2

LeBauer, D.S., Treseder, K.K., 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. Ecology 89, 371–379. https://doi.org/10.1890/06-2057.1

Liao, P., Huang, S., van Gestel, N.C., Zeng, Y., Wu, Z., van Groenigen, K.J., 2018. Liming and straw retention interact to increase nitrogen uptake and grain yield in a double rice-cropping system. F. Crop. Res. 216, 217–224. https://doi.org/10.1016/j.fcr.2017.11.026

Lin, G., Guo, D., Li, L., Ma, C., Zeng, D.H., 2018. Contrasting effects of ectomycorrhizal and arbuscular mycorrhizal tropical tree species on soil nitrogen cycling: the potential mechanisms and corresponding adaptive strategies. Oikos 127, 518–530. https://doi.org/10.1111/oik.04751

Lü, X.T., Dijkstra, F.A., Kong, D.L., Wang, Z.W., Han, X.G., 2014. Plant nitrogen uptake drives responses of productivity to nitrogen and water addition in a grassland. Sci. Rep. 4, 1–7. https://doi.org/10.1038/srep04817

Maeda, Y., Tashiro, N., Enoki, T., Urakawa, R., Hishi, T., 2018. Effects of species replacement on the relationship between net primary production and soil nitrogen availability along a topographical gradient: Comparison of belowground allocation and nitrogen use efficiency between natural forests and plantations. For. Ecol. Manage. 422, 214–222.

https://doi.org/10.1016/j.foreco.2018.03.046

Marschner, H., Dell, B., 1994. Nutrient uptake in mycorrhizai symbiosis. Plant Soil 159, 89–102. https://doi.org/10.1007/BF00000098

Meier, I.C., Finzi, A.C., Phillips, R.P., 2017. Root exudates increase N availability by stimulating microbial turnover of fast-cycling N pools. Soil Biol. Biochem. 106, 119–128. https://doi.org/10.1016/J.SOILBIO.2016.12.004

Meier, I.C., Pritchard, S.G., Brzostek, E.R., Mccormack, M.L., Phillips, R.P., 2015. The rhizosphere and hyphosphere differ in their impacts on carbon and nitrogen cycling in forests exposed to elevated CO2. New Phytol. 205, 1164–1174. https://doi.org/10.1111/nph.13122

Meier, I.C., Tückmantel, T., Heitkötter, J., Müller, K., Preusser, S., Wrobel, T.J., Kandeler, E., Marschner, B., Leuschner, C., 2020. Root exudation of mature beech forests across a nutrient availability gradient: the role of root morphology and fungal activity. New Phytol. 226, 583–594. https://doi.org/10.1111/nph.16389

Miranda, K.M., Espey, M.G., Wink, D.A., 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide - Biol. Chem. 5, 62–71. https://doi.org/10.1006/niox.2000.0319

Moreau, D., Pivato, B., Bru, D., Busset, H., Deau, F., Faivre, C., Matejicek, A., Strbik, F., Philippot, L., Mougel, C., 2015. Plant traits related to nitrogen uptake influence plant-microbe competition. Ecology 96, 2300–2310. https://doi.org/10.1890/14-1761.1

Nakayama, M., Tateno, R., 2018. Solar radiation strongly influences the quantity of forest tree root exudates. Trees 32, 871–879. https://doi.org/10.1007/s00468-018-1685-0

Nakayama, M., Imamura, S., Taniguchi, T., Tateno, R., 2019. Does conversion from natural forest to plantation affect fungal and bacterial biodiversity, community structure, and co-occurrence networks in the organic horizon and mineral soil? For. Ecol. Manage. 446, 238–250.

https://doi.org/10.1016/j.foreco.2019.05.042

Obase, K., Tamai, Y., Yajima, T., Miyamoto, T., 2007. Mycorrhizal associations in woody plant species at the Mt. Usu volcano, Japan. Mycorrhiza 17, 209–215. https://doi.org/10.1007/s00572-006-0097-y

Oberson, A., Friesen, D.K., Rao, I.M., Bühler, S., Frossard, E., 2001. Phosphorus transformations in an Oxisol under contrasting land-use systems: The role of the soil microbial biomass. Plant Soil 237, 197–210. https://doi.org/10.1023/A:1013301716913

Ohsawa, T., Tsuda, Y., Saito, Y., Ide, Y., 2011. The genetic structure of Quercus crispula in northeastern Japan as revealed by nuclear simple sequence repeat loci. J. Plant Res. 124, 645–654. https://doi.org/10.1007/s10265-010-0402-x

Phillips, R.P., Erlitz, Y., Bier, R., Bernhardt, E.S., 2008. New approach for capturing soluble root exudates in forest soils. Funct. Ecol. 22, 990–999. https://doi.org/10.1111/j.1365-2435.2008.01495.x

Phillips, R.P., Fahey, T.J., 2006. Tree species and mycorrhizal associations influence the magnitude of rhizosphere effects. Ecology 87, 1302–1313. https://doi.org/10.1890/0012-9658(2006)87[1302:TSAMAI]2.0.CO;2

Phillips, R.P., Fahey, T.J., 2008. The influence of soil fertility on rhizosphere effects in northern hardwood forest soils. Soil Sci. Soc. Am. J. 72, 453–461. https://doi.org/10.2136/sssaj2006.0389

Phillips, R.P., Finzi, A.C., Bernhardt, E.S., 2011. Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO2 fumigation. Ecol. Lett. 14, 187–194. https://doi.org/10.1111/j.1461-0248.2010.01570.x

Qing, Q., Yang, B., Wyman, C.E., 2010. Xylooligomers are strong inhibitors of cellulose hydrolysis by enzymes. Bioresour. Technol. 101, 9624–9630. https://doi.org/10.1016/j.biortech.2010.06.137

R Core Team, 2018. R: A language and environment for statistical computing. R Foundation for

691 Statistical Computing, Vienna, Austria. URL https://www.R-project.org/
692 Reich, P.B., Grigal, D.F., Aber, J.D., Gower, S.T., 1997. Nitrogen mineralization and productivity in 50
693 hardwood and conifer stands on diverse soils. Ecology 78, 335–347. https://doi.org/10.1890/0012-
694 9658(1997)078[0335:NMAPIH]2.0.CO;2
695 Saiya-Cork, K.R., Sinsabaugh, R.L., Zak, D.R., 2002. The effects of long term nitrogen deposition on
696 extracellular enzyme activity in an Acer saccharum forest soil. Soil Biol. Biochem. 34, 1309–1315.
697 https://doi.org/10.1016/S0038-0717(02)00074-3
698 Shahzad, T., Chenu, C., Genet, P., Barot, S., Perveen, N., Mougin, C., Fontaine, S., 2015. Contribution of
699 exudates, arbuscular mycorrhizal fungi and litter depositions to the rhizosphere priming effect
700 induced by grassland species. Soil Biol. Biochem. 80, 146–155.
701 https://doi.org/10.1016/j.soilbio.2014.09.023
702 Shibata, H., Urakawa, R., Toda, H., Inagaki, Y., Tateno, R., Koba, K., Nakanishi, A., Fukuzawa, K.,
703 Yamasaki, A., 2011. Changes in nitrogen transformation in forest soil representing the climate
704 gradient of the Japanese archipelago. J. For. Res. 16, 374–385. https://doi.org/10.1007/s10310-011-
705 0288-z
706 Soudzilovskaia, N.A., van der Heijden, M.G.A., Cornelissen, J.H.C., Makarov, M.I., Onipchenko, V.G.,
707 Maslov, M.N., Akhmetzhanova, A.A., van Bodegom, P.M., 2015. Quantitative assessment of the
708 differential impacts of arbuscular and ectomycorrhiza on soil carbon cycling. New Phytol. 208,
709 280–293. https://doi.org/10.1111/nph.13447
710 Takagi, K., Kotsuka, C., Fukuzawa, K., Kayama, M., Makoto, K., Watanabe, T., Nomura, M., Fukazawa,
711 T., Takahashi, H., Hojyo, H., Ashiya, D., Naniwa, A., Sugata, S., Kamiura, T., Sugishita, Y., Sakai,
712 R., Ito, K., Kobayashi, M., Maebayashi, M., Mizuno, M., Murayama, T., Kinoshita, K., Fujiwara,
713 D., Hashida, S., Shibata, H., Yoshida, T., Sasa, K., Saigusa, N., Fujinuma, Y., Akibayashi, Y.,
714 2010. Allometric Relationships and Carbon and Nitrogen Contents for Three Major Tree Species

(Quercus crispula, Betula ermanii, and Abies sachalinensis) in Northern Hokkaido, Japan. Eurasian J. For. Res. 13, 1–7.

Talbot, J.M., Treseder, K.K., 2010. Controls over mycorrhizal uptake of organic nitrogen. Pedobiologia 53, 169–179. https://doi.org/10.1016/j.pedobi.2009.12.001

Tateno, R., Takeda, H., 2010. Nitrogen uptake and nitrogen use efficiency above and below ground along a topographic gradient of soil nitrogen availability. Oecologia 163, 793–804. https://doi.org/10.1007/s00442-009-1561-0

Tateno, R., Nakayama, M., Yano, M., Fukuzawa, K., Inagaki, Y., Koba, K., Ugawa, S., 2020. Nitrogen source utilization in co-existing canopy tree and dwarf bamboo in a northern hardwood forest in Japan. Trees 34, 1047–1057. https://doi.org/10.1007/s00468-020-01980-1

Tietema, A., Warmerdam, B., Lenting, E., Riemer, L., 1992. Abiotic factors regulating nitrogen transformations in the organic layer of acid forest soils: Moisture and pH. Plant Soil 147, 69–78. https://doi.org/10.1007/BF00009372

Toljander, J.F., Lindahl, B.D., Paul, L.R., Elfstrand, M., Finlay, R.D., 2007. Influence of arbuscular mycorrhizal mycelial exudates on soil bacterial growth and community structure. FEMS Microbiol. Ecol. 61, 295–304. https://doi.org/10.1111/j.1574-6941.2007.00337.x

Urakawa, R., Shibata, H., Kuroiwa, M., Inagaki, Y., Tateno, R., Hishi, T., Fukuzawa, K., Hirai, K., Toda, H., Oyanagi, N., Nakata, M., Nakanishi, A., Fukushima, K., Enoki, T., Suwa, Y., 2014. Effects of freeze-thaw cycles resulting from winter climate change on soil nitrogen cycling in ten temperate forest ecosystems throughout the Japanese archipelago. Soil Biol. Biochem. 74, 82–94. https://doi.org/10.1016/j.soilbio.2014.02.022

Uren, N., 2007. Types, Amounts, and Possible Functions of Compounds Released into the Rhizosphere by Soil-Grown Plants, in: Pinton, R., Varanini, Z., Nannipieri, P. (Eds.), The Rhizosphere: Biochemistry and organic substances at the soil-plant interdace, second edition. CRC Press, pp. 1–

21. https://doi.org/10.1201/9781420005585.ch1

van Hees, P.A., Rosling, A., Essén, S., Godbold, D.L., Jones, D.L., Finlay, R.D., 2006. Oxalate and ferricrocin exudation by the extramatrical mycelium of an ectomycorrhizal fungus in symbiosis with Pinus sylvestris. New Phytol. 169, 367–378. https://doi.org/10.1111/j.1469-8137.2005.01600.x

Vitousek, P.M., Gosz, J.R., Grier, C.C., Melillo, J.M., Reiners, W.A., Todd, R.L., 1979. Nitrate losses from disturbed ecosystems. Science. 204, 469–474. https://doi.org/10.1126/science.204.4392.469

Vitousek, P., Howarth, R., 1991. Nitrogen limitation on land and in the sea: How can it occur? Biogeochemistry 13, 87–115. https://doi.org/10.1007/BF00002772

Watanabe, T., Tateno, R., Imada, S., Fukuzawa, K., Isobe, K., Urakawa, R., Oda, T., Hosokawa, N., Sasai, T., Inagaki, Y., Hishi, T., Toda, H., Shibata, H., 2019. The effect of a freeze–thaw cycle on dissolved nitrogen dynamics and its relation to dissolved organic matter and soil microbial biomass in the soil of a northern hardwood forest. Biogeochemistry 142, 319–338. https://doi.org/10.1007/s10533-019-00537-w

Yan, E.R., Wang, X.H., Huang, J.J., Li, G.Y., Zhou, W., 2008. Decline of soil nitrogen mineralization and nitrification during forest conversion of evergreen broad-leaved forest to plantations in the subtropical area of Eastern China. Biogeochemistry 89, 239–251. https://doi.org/10.1007/s10533-008-9216-5

Yin, H., Li, Y., Xiao, J., Xu, Z., Cheng, X., Liu, Q., 2013. Enhanced root exudation stimulates soil nitrogen transformations in a subalpine coniferous forest under experimental warming. Glob. Chang. Biol. 19, 2158–2167. https://doi.org/10.1111/gcb.12161

Yin, H., Wheeler, E., Phillips, R.P., 2014. Root-induced changes in nutrient cycling in forests depend on exudation rates. Soil Biol. Biochem. 78, 213–221. https://doi.org/10.1016/j.soilbio.2014.07.022

Zhu, B., Gutknecht, J.L.M., Herman, D.J., Keck, D.C., Firestone, M.K., Cheng, W., 2014. Rhizosphere priming effects on soil carbon and nitrogen mineralization. Soil Biol. Biochem. 76, 183–192.

https://doi.org/10.1016/J.SOILBIO.2014.04.033

Zou, X., Valentine, D.W., Sanford, R.L., Binkley, D., 1992. Resin-core and buried-bag estimates of nitrogen transformations in Costa Rican lowland rainforests, Plant Soil. 139, 275–283 https://doi.org/10.1007/BF00009319

767 Table 1 Total C and N contents and C/N ratios of the mineral soil and the organic horizon used in the incubation.

	Total C (%)	Total N (%)	C/N ratio	Dry weight used in the incubation (g pipe^{-1})
Organic horizon				
Relatively fresh	48.94 ± 0.75	1.10 ± 0.14	45.0 ± 5.8	0.25
Partially decomposed	45.71 ± 1.96	1.26 ± 0.21	37.4 ± 7.3	2.46
Passed through 9 mm mesh	42.75 ± 3.16	1.67 ± 0.11	25.6 ± 1.2	1.25
Mineral soil	8.58 ± 0.99	0.59 ± 0.07	14.6 ± 0.3	129.64

768 Note: Values represent the mean ± standard deviation.

769 Table 2 Equations used to estimate nitrogen uptake, nitrogen mineralization, nitrification, and the priming effect on nitrogen mineralization.

Parameter	Abbreviation	Equation
Nitrogen uptake		
Nitrogen demand of *Q. crispula*	N_{demand}	Sum of nitrogen contents in each part of net primary production
Potential nitrogen uptake per dry weight of fine roots under natural conditions	Nat N_{uptake}	N_{demand} / Fine root density of *Q. crispula* (0–50cm) in study plots
Potential nitrogen uptake by fine roots in incubation pipes	N_{uptake}	Nat N_{uptake} × Fine root densities in incubation pipes
Nitrogen dynamics		
Net nitrate increment	Nit_{inc}	Annual leaching + increment of pools of NO_3^--N
Net inorganic nitrogen increment	N_{inc}	Annual leaching + increment of pools of NO_3^--N and NH_4^+-N
Fine root induced increment of inorganic nitrogen	$N_{induced}$	$N_{min,R} - N_{min,C} + N_{uptake}$

770 Note: $N_{min,R}$ and $N_{min,C}$ represent N_{min} of samples with and without *Q. crispula* fine roots, respectively.

771

772

773 Table 3 Production, N concentration, and N demand for *Q. crispula* overall and for individual plant parts.

	Production ($g/m^2/year$)	N concentrations (%)	Estimated N demand ($g\text{-}N/m^2/year$)
Total NPP	601.5 ± 76.5		6.90 ± 1.01
Leaf litter	256.7 ± 36.1	1.82 ± 0.08	4.67 ± 0.66
Other litter	21.2 ± 9.14	1.46	0.31 ± 0.13
Branch	96.3 ± 11.7[*1]	0.58[*2]	0.56 ± 0.07
Trunk	152.2 ± 23.0[*1]	0.39[*2]	0.59 ± 0.09
Coarse root	62.2 ± 10.2[*1]	0.45[*2]	0.28 ± 0.05
Fine root (0-10cm)	18.3 ± 23.5	1.43 ± 0.16	0.26 ± 0.34
Fine root (0-50cm)	34.1 ± 43.8[*3]		0.49 ± 0.63[*3]

774 Note: NPP represents the net primary production. Values represent the mean ± standard deviation (n = 4 for the production of branch, trunk, and coarse roots,

775 and the N concentration in fine roots; n = 8 for leaf litter; n = 32 for fine root production).

776 *1: Branch, trunk, and coarse root productions were calculated based on the allometric relationship (Takagi et al., 2010) and increase in diameters at breast

777 height.

778 *2: N concentrations in the branch, trunk, and coarse root were based on a previous study (Takagi et al., 2010).

779 *3: Fine-root production (0–50 cm) was estimated by the vertical distribution of the fine roots of *Q. crispula* in the adjacent plot (Tateno et al., 2020).

780

781 Table 4 Net N dynamics, estimated potential root N uptake based on the root density in the modified resin core, and the root-induced annual inorganic N

782 increment (0–10 cm soil depth).

	With organic horizon		Without organic horizon	
	With root	Without root	With root	Without root
Net N dynamics				
Nit_{inc}(g-N m^{-2} $year^{-1}$)	6.61 ± 1.04	7.14 ± 1.09	4.93 ± 0.90	4.99 ± 1.13
N_{inc}(g-N m^{-2} $year^{-1}$)	6.53 ± 1.11	7.01 ± 1.12	4.78 ± 0.87	4.92 ± 1.11
N_{uptake} (g-N m^{-2} $year^{-1}$)	0.52 ± 0.18		0.53 ± 0.13	
$N_{induced}$ (g-N m^{-2} $year^{-1}$)	0.04 ± 0.55		0.32 ± 0.48	

783 Note: N_{nit}, N_{min}, N_{uptake}, and N_{primed} represent the annual nitrification, annual nitrogen mineralization, annual nitrogen uptake, and annual primed nitrogen

784 mineralization by the fine roots in the incubation pipes, respectively. The values represent mean ± standard deviation.

785