The Revision for *GEODER 2018 1924*

Increasing soil age drives shifts in plant-plant interactions from positive to negative and affects primary succession dynamics in a subalpine glacier forefield

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Highlights

- Facilitation frequently occurring in subalpine environments promotes survival.
- *Populus-Salix* interplay switches from positive to negative during primary succession.
- Soil age regulates plant-plant interactions in a subalpine glacier forefield.
- Nitrogen availability mediates plant-soil feedbacks between neighboring plants.

Abstract The stress gradient hypothesis predicts that plant-plant interactions switch between facilitation (positive) and competition (negative) along environmental gradients, with facilitation being more common under high abiotic stress conditions relative to more moderate abiotic stress conditions. Our aim was to reveal, whether the interactions between *Populus purdomii* Rehder and *Salix rehderiana* Schneider switch from positive to negative during the early stages of primary succession in the Gongga Mountain glacier retreat region. We also investigated, whether soil age is a major driving factor for the transformation of interactions between neighboring plants. We analyzed differences between intraspecific interactions and interspecific interactions of *Populus* and *Salix* under 20- and 40-year-old soil conditions, including plant biomass accumulation and allocation, nutrient absorption and utilization, relative competition intensity, non-structural carbohydrates, foliar carbon and nitrogen isotope composition, mesophyll cell ultrastructure, soil microbial biomass and community structure, extracellular enzyme activities, and soil organic carbon (SOC), soil total nitrogen (TN), soil ammonium (NH_4^+-N), and soil nitrate (NO_3^--N) contents. We found that *P. purdomii* and *S. rehderiana* growing under interspecific interactions had greater contents of aboveground dry matter, belowground dry matter and total dry matter compared to intraspecific interactions in 20-year-old soil. Furthermore, in 40-year-old soil conditions, the phospholipid fatty acid (PLFA) analysis showed that *Populus* and *Salix* exposed to interspecific interactions exhibited lower amounts of gram-positive bacteria, fungi (18:1 ω9c) and actinomycetes, and lower levels of total PLFAs than those growing under intraspecific interactions. The redundancy analysis (RDA) results demonstrated that soil N was the most important parameter contributing to the composition of microbial communities. In addition, the ^{15}N stable isotope labeling method showed that *Populus* and *Salix* growing under interspecific interactions had higher foliage $\delta^{15}N$

derived from NO_3^- ($\delta^{15}N\text{-}NO_3^-$) than those growing under intraspecific interactions in 20-year-old soil. In summary, our results demonstrated that *Populus*-*Salix* interactions exhibited positive effects on survival in 20-year-old soil. Conversely, under 40-year-old soil conditions, *Populus*-*Salix* interactions presented negative effects in relation to nutrients and elimination by neighboring plants. Moreover, soil age is a major driving factor for plant-plant interactions that shift from positive to negative with an increasing soil age in the Gongga Mountain glacier retreat area. In all, our results support the stress gradient hypothesis. Our findings improve understanding of plant-plant interactions and plant-soil feedbacks during the early stages of soil development, and of the construction of vegetation communities.

Keywords: Plant-plant interactions; Positive and negative effects; ^{13}C and ^{15}N stable isotope composition; Microbial community structure; Primary succession; Glacier retreat area.

1. Introduction

The stress gradient hypothesis (SGH) predicts that the dynamic balance between facilitation and competition varies along environmental gradients, with facilitation dominating under high environmental stress and competition under low environmental stress (Bertness and Callaway, 1994; Travis et al., 2006; He et al., 2013). Experiments that have provided support for the stress gradient hypothesis have demonstrated that the impact of facilitation increases relative to competition with increasing abiotic stress (Callaway et al., 2002; Dohn et al., 2013; Michalet et al., 2014). Switching between facilitation and competition could be primarily attributed to changes in external abiotic and biotic conditions (e.g., changes in light, water, space, temperature, soil quality and nutrient availability).

Plant-plant competitive interactions influence plant growth, distribution and abundance, species coexistence, and the composition of plant communities in terrestrial ecosystems (Connell, 1983; Choler et al., 2001; Brooker, 2006). Recent studies have focused on plant-plant facilitative interactions that regulate individual fitness, species composition and diversity, and vegetation community structures, particularly those in alpine ecosystems (Lin et al., 2012; Arroyo et al., 2015; Kéfi et al., 2016; Qi et al., 2018). Some studies have shown that facilitative interactions mainly occur at high elevations and under high biotic and abiotic environmental stress conditions (Choler et al., 2001; Bruno et al., 2003; Kikvidze et al., 2006). Competition and facilitation are major drivers of plant community structure and composition, as well as plant species dynamics (Fowler, 1986; Choler et al., 2001).

Morphological and physiological traits respond to plant-plant interactions (positive or negative), as well as soil carbon:nitrogen (C:N) and nitrogen:phosphorus (N:P) ratios, with repercussions on soil microbial communities and soil fertility (Huston and DeAngelis, 1994; Callaway et al., 1997; Zechmeister-Boltenstern et al., 2015). Jiang et al. (2018) showed that the leaf N:P ratio increased steadily in a glacier retreat area, and the limiting factor for plant growth shifted from nitrogen to phosphorus. Plants have developed different physiological responses to environmental stresses (such as drought, nutrient deficiency or competition): they perceive and transmit stress signals, as well as regulate morphology and physiological properties (Chen et al., 2010; Zhang et al., 2014; Chen et al., 2015). Furthermore, aboveground and belowground subsystems and their feedbacks are important processes for studying the relationships of neighboring plants (Wardle et al., 2004; Ushio et al., 2016).

Plant-soil microbes associations are an important link between aboveground and belowground subsystems. For instance, plant residues (e.g., leaf litter, woody debris and dead roots) affect microbial activities and serve as a major source of carbon and nutrients for microbial decomposer communities (Chapin et al., 1994; Kaye et al., 1997; van der Heijden et al., 2003). Meanwhile, the chemical composition of plant residues affects microbial activities and community structures and, in turn, soil microorganisms are primary decomposers of dead plant material and they influence plants' nutritional quality (Aneja et al., 2006; Göransson et al., 2011). Aboveground and belowground interactions drive the structure and functioning of ecosystems, including carbon and nutrient cycling (Kardol and Wardle, 2010).

During primary succession in a glacier forefield, changes in the biochemical properties of soil (e.g., increasing nutrient availability and accumulation of organic matter) and in the composition of microbial communities influence plant growth and performance traits (Hodkinson et al., 2003; Walker et al., 2010; Castle et al., 2016). Nutrient resources are limited during the early stages of primary succession (He and Tang, 2008; Göransson et al., 2011). Low nutrient availability frequently limits plant growth and competitive ability, particularly in cold ecosystems, such as alpine or subalpine environments, where low temperature restricts underground decomposition processes of soil organic matter (Chapin and Shaver, 1985; Nadelhoffer et al., 1991; Robinson et al., 1995). The decomposition of plant litter is closely related to soil nutrient (N and P) contents, and soil microbes are crucial for plant litter decomposition (Fujii and Takeda, 2010; Zhao et al., 2013; Zechmeister-Boltenstern et al., 2015).

Primary succession in the Gongga Mountain glacier forefield has resulted in the establishment of following predominant plant populations: bare land, *Astragalus mahoshanicus* and *Epilobium amurense* herb vegetation, *Hippophaer hamnoides* and *Salix rehderiana* sapling scrub forest, *Salix rehderiana* and *Populus purdomii* deciduous broad-leaved forest, *Betulautilis* and *Rhododendron simsii* evergreen broad-leaved forest, and *Abies fabri* and *Picea brachytyla* evergreen coniferous forest (Zhou et al., 2013; Lei et al., 2015). During primary succession, *S. rehderiana* initially appears, then *P. purdomii*, and later, *P. purdomii* replaces *S. rehderiana* as the dominant trees in the community, followed by multiple interactive relationships between *P. purdomii* and *S. rehderiana*. Soil nitrogen, which plays a key role in plant growth, is limited during the early primary succession

123 (Chapin et al., 1994; He and Tang, 2008). Previous studies have shown that litter decomposition by
124 N-fixing species contributes to the accumulation of soil N during succession, with the topsoil having
125 the highest amounts of nutrients (Jia et al., 2005). Song et al. (2017) showed that N-addition could
126 regulate changes in the competitive ability between *P. purdomii* and *S. rehderiana*. However, our
127 understanding of the major effects of soil age on plant-plant interactions and plant-soil feedbacks
128 during soil development and primary succession in a glacial forefield, especially considering
129 climate warming, is limited.
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131 In the present study, we examined the relationship between *P. purdomii* and *S. rehderiana*
132 individuals (e.g., from positive to negative, from negative to positive, or no change) along with
133 aging soil under intra- and interspecific interactions in the Gongga Mountain glacier retreat area.
134 Moreover, soil microbial community structures and soil nutrient availabilities were measured in
135 order to elucidate the mechanism of the switch between facilitation and competition. We
136 hypothesized the following: 1) plant-plant interactions predominantly show facilitation in severe
137 environments during the early stages of primary succession in subalpine glacier retreat areas,
138 whereas under relatively benign nutrient conditions, the interactions between neighboring plants
139 change into competition; 2) the composition of soil microbes and nutrient abundance are the main
140 driving forces for the shift of *Populus*-*Salix* interactions from positive to negative with increasing
141 soil age. To validate these hypotheses, we examined a set of morphological and physiological
142 indexes of plants, soil microbial biomass and community composition, extracellular enzyme
143 activities, and nutrient availability.
144

2. Materials and methods

2.1. Study site

The experiment was conducted at the Gongga Mountain Alpine Ecosystem Observation and Experiment Station of the Chinese Academy of Sciences (29°34' N, 101°59' E, 3,000 m a.s.l.), located on the south-eastern fringe of the Tibetan Plateau (Fig. 1). The mean annual temperature is 4.2°C, mean annual precipitation is approximately 1,949 mm (rainfall mainly occurring from June to September), and mean annual air relative humidity is about 90.2% (Yang et al., 2014). The Hailuogou glacier is one of the most representative monsoonal temperate valley glaciers. Its length is around 2,000 m, width about 50-200 m and elevation between 2,850-3,000 m (He and Tang, 2008; Liu et al., 2010). It is located on the eastern slope of the Gongga Mountain, with a glacier area of approximately 25 km^2 (Liu et al., 2010). During recent years, the Hailuogou glacier has decreased in size due to climate warming. During the past 120 years, the glacier retreat area has undergone a long-term primary succession from pioneer species to an evergreen coniferous forest following a chronosequence of soil development. Previously, this glacier forefield has been utilized when studying primary succession, soil formation processes, plant-soil feedbacks and plant-plant interactions (Wang et al., 2016; Song et al., 2017; Jiang et al., 2018; Jiang et al., 2019).

2.2. Plant materials and experimental design

Current season seedlings of *P. purdomii* and *S. rehderiana* were selected from the nursery

surrounding the station in September 2014. A total of 72 seedlings were collected (36 of each species). The seedlings had uniform basal stem diameter (about 3.5 mm) and height (about 15 cm). Two seedlings (situated 10 cm apart) were planted into a plastic pot (internal diameter: 30 cm; height: 25 cm) with 20- and 40-year-old soil (two seedlings per pot; two seedlings of *P. purdomii* or *S. rehderiana*, or one *P. purdomii* and one *S. rehderiana*). The 20- and 40-year-old soils used in the experiments were collected from the topsoil (0-15 cm) in the glacier retreat area. The 20- and 40-year-old soils had soil organic carbon contents of 11.81 $g \cdot kg^{-1}$ and 16.59 $g \cdot kg^{-1}$, total nitrogen of 0.34 $g \cdot kg^{-1}$ and 0.54 $g \cdot kg^{-1}$, total phosphorus of 0.34 $g \cdot kg^{-1}$ and 0.29 $g \cdot kg^{-1}$, and total potassium of 6.62 $g \cdot kg^{-1}$ and 10.73 $g \cdot kg^{-1}$, respectively. The experimental layout was completely randomized with three factors (species, soil age and planting pattern). Experiments with two species (*P. purdomii* and *S. rehderiana*), two soil ages (20 and 40 years old), and three planting patterns (*P. purdomii* and *P. purdomii* intraspecific treatment; *S. rehderiana* and *S. rehderiana* intraspecific treatment; and *P. purdomii* and *S. rehderiana* interspecific treatment) were established (Fig. S1). Six replicates per treatment were included in the study. The experiments were performed on an open site in the field under natural rain conditions. Furthermore, we used the ^{15}N isotope tracer method to study differences in the absorption and utilization of N forms (NH_4^+ and NO_3^-) in *P. purdomii* and *S. rehderiana* individuals. The ^{15}N tracer solution, with labelled $^{15}NH_4NO_3$ and $NH_4{}^{15}NO_3$, was injected into the soil at a depth of 5 cm around the plants (30 mg $^{15}NH_4^+$-N per plant or 30 mg $^{15}NO_3^-$-N mg per plant). Then, 72 h after the application of the ^{15}N solution, the plants were harvested and foliage ^{15}N values were determined (Chen et al., 2014).

2.3. Morphological and physiological indexes

Four pots from six replicates were randomly harvested at the end of the experiment on August 25, 2015. All individuals were sorted into roots, stems and leaves, and all biomass samples were dried (70°C, 72 h) to a constant weight to measure aboveground, belowground, and total dry matter accumulation. The root to shoot (R/S) ratios were calculated. Dried leaf and root samples were ground to a fine powder and were passed through a mesh (pore diameter: 250 μm). Then, the C, N and P contents of these tissues were measured by the rapid dichromate oxidation technique (Nelson and Sommers, 1982), the semi-micro Kjeldahl method (Fawcett, 1954), and induced plasma emission spectroscopy (Parkinson and Allen, 1975), respectively.

Non-structural carbohydrates of the root and leaf tissues were estimated as starch, soluble sugar, fructose, and sucrose contents. Approximately 50 mg of each dry powdered plant sample was mixed with 6 mL of 80% (v/v) ethanol, incubated for 30 min in a water bath at 80°C, then centrifuged for 5 min at 7,000 *g* (Guo et al., 2016; Song et al., 2017). The ethanol extract solution was used for the determination of the fructose content according to a modified resorcinol method (Murata et al., 1968), the sucrose content was measured according to the procedure of Green et al. (1994), and the total soluble sugar content was measured according to the anthrone-sulfuric acid method (Yemm and Willis, 1954). The solid residue was hydrolyzed with 2 mL of 9.2 $mol \cdot L^{-1}$ $HClO_4$ for 30 min to measure the starch content (Yemm and Willis, 1954).

2.4. Foliage C and N isotope composition

Stable isotope analyses were performed at the Stable Isotope Laboratory, Chinese Academy of Forestry, using a DELTA V Advantage Isotope Ratio Mass Spectrometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA). The carbon isotope composition ($\delta^{13}C$) was used to estimate long-term tree water use efficiency (Dong et al., 2015). The $\delta^{13}C$ value of the samples was expressed relative to the standard Pee Dee Belemnite (Farquhar et al., 1989) as follows: $\delta^{13}C$ (‰) = ($R_{sample}/R_{standard}$ - 1) × 1,000, where R_{sample} is the $^{13}C/^{12}C$ ratio of the sample, and $R_{standard}$ is that of the standard substance. The ^{15}N concentration values were converted to $\delta^{15}N$ using the following equation: $\delta^{15}N$ (‰) = ($T_{sample}/T_{standard}$ - 1) × 1,000, where T_{sample} is the $^{15}N/^{14}N$ ratio of the sample, and $T_{standard}$ is that of the standard substance. The overall precision of the $\delta^{13}C$ and $\delta^{15}N$ estimations was better than 0.1‰, as determined from four replicates in each case.

2.5. Leaf ultrastructural assessment

Mesophyll cell observations are widely used to investigate changes in leaf organelles under various abiotic stresses (e.g., competitive pressure or nutrient deficiency). Sections (2 mm in length) from middle leaf parts were selected for transmission electron microscopy (TEM) analyses according to the procedures of Zhao et al. (2009) and Song et al. (2017). In brief, the sections were fixed in 3% glutaraldehyde (v/v) in 0.2 M sodium phosphate buffer (pH 7.2) for 6-8 h, post-fixed in 1% osmium tetroxide for 1 h, and finally, again, in 0.2 M sodium phosphate buffer (pH 7.2) for 1-2 h. Ultrathin sections (80 nm thick) were sliced, stained with uranyl acetate and lead citrate, and mounted on copper grids for viewing on H-600IV TEM (Hitachi, Tokyo, Japan).

233 *2.6. Soil sampling and biochemical analysis*

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235 Four soil samples from each treatment were randomly selected at the end of the experiment to
236 analyze soil biochemical properties and microbial community structures. Topsoil samples were
237 collected from depths of 0-15 cm around the seedlings, immediately transported to the laboratory for
238 further analysis and stored at 4 °C for a later assessment of microbial biomass and enzyme activities,
239 and at -20 °C for a subsequent phospholipid fatty acid analyses (performed within one week). The
240 moist soil samples were sieved (pore size: 2 mm) to remove large organic debris prior to analyses.
241 The remaining soil samples were air-dried at room temperature and used for the determination of
242 chemical properties.

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244 Soil organic C concentrations were measured using the potassium dichromate oxidation-ferrous
245 sulfate titrimetry method (Nelson and Sommers, 1982). Total N concentrations were determined
246 using a LECO EPS-2000 CNS thermal combustion furnace (LECO Corp., St Jose, MI). Total P was
247 melted by sodium carbonate, and determined by molybdenum-blue colorimetry. Total potassium (K)
248 was digested by HF-$HClO_4$ and detected by flame photometry. Alkali-hydrolyzable N was extracted
249 with 2 M KCl and measured using a microplate reader (Biotek, Winooski, USA). Available P was
250 extracted with 0.5 M $NaHCO_3$ and determined using the molybdenum blue method. Available K
251 was extracted with 1 M ammonium acetate and quantified by flame photometry (FP640, INASA,
252 China). Soil pH was determined using a 1:2.5 ratio of soil (in grams) to water (in milliliters) using a
253 pH electrode (FE20, Mettler Toledo, Switzerland). Ammonium (NH_4^+-N) and nitrate (NO_3^--N) were
254 extracted with 2 M KCl, and determined using an autoanalyser (SEAL-AA3, Germany). Microbial

biomass carbon (MBC) and nitrogen (MBN) were measured using fresh soil and the chloroform fumigation-extraction method (Brookes et al. 1985; Vance et al. 1987) with a Multi-N/C2100 analyzer (Analytik Jena, Germany). MBC and MBN were calculated from the differences between extractable C and N concentrations in fumigated and unfumigated samples using conversion factors (k_{EC} and k_{EN}) of 0.45 for both (Vance et al. 1987). The urease activity was measured by colorimetry according to the method of Kandeler and Gerber (1988) and was expressed as μg NH_4-N·g^{-1}·soil·h^{-1}. The nitrate reductase activity was determined using the colorimetric method (Kandeler, 1996) and was expressed as μg NO_2-N·g^{-1}·soil·h^{-1}. The saccharase activity was determined with a spectrophotometer (U-2800, Japan) at a wavelength of 508 nm by the method described by Guan (1986) and was expressed as μg glucose·g^{-1}·soil·h^{-1}.

2.7. Phospholipid fatty acid extraction and analysis

The composition of soil microbial communities was assessed by the PLFA analysis using a modified method of Frostegård et al. (1991), as described by Xu et al. (2015) and Duan et al. (2015). Briefly, 6 g of frozen soil was extracted with a chloroform:methanol:citrate acid buffer mixture (1:2:0.8, v/v/v). The lipids were separated into neutral lipids, glycolipids, and phospholipids on silicic acid columns. The phospholipids were subjected to mild alkaline methanolysis (Schindlbacher et al., 2011). The extracted fatty acid methyl esters were analyzed using a Hewlett-Packard 6890 gas chromatograph with an Ultra 2-HP capillary column (cross-linked with 5% phenyl-methyl silicone; 25 m, 0.22 mm ID, and 0.33 μm thickness). The peaks were identified using bacterial fatty acids as standards and utilizing the Sherlock peak identification software (MIDI, Inc. Newark, DE, USA).

277 Fatty acids were quantified through a comparison of the sample peak areas with those of internal
278 standards (19:0 nonadecanoic methyl ester) (Smithwick et al., 2005). The areas measured by
279 GC-FID were used to calculate the abundance of PLFA markers, which were expressed as
280 nmol·PLFA g^{-1} dry soil (Schindlbacher et al., 2011). We used the terminal-branched saturated PLFA
281 peaks i14:0, a15:0, i15:0, i15:0 G, i16:0, i17:0, and a17:0 as markers for gram-positive (G+) bacteria
282 (Zelles, 1997). The mono-unsaturated and cyclopropyl-saturated peaks 16:1 2OH, 17:1ω8c,
283 18:1ω5c, cy17:0, and cy19:0ω8c were used as markers for gram-negative (G-) bacteria (Federle,
284 1986; Frostegård et al., 2011). The 18:1ω9c marker was used as an indicator of fungi, whereas
285 16:1ω5c and 16:1ω11c were used as representatives of arbuscular mycorrhizal fungi (Bossio et al.,
286 1998; Swallow et al., 2009; Huang et al., 2013; Xu et al., 2015). The PLFA peaks 10 Me17:0 and 10
287 Me18:0 were selected as indicators of actinomycetes (Waldrop et al., 2004).
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289 *2.8. Data analyses*
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291 The experimental layout was completely randomized with three factors (species, planting pattern
292 and soil age). Experiments with *P. purdomii* and *S. rehderiana* were established to test intraspecific
293 interactions (two of the same species per mesocosm) or interspecific interactions (two different
294 species per mesocosm) in soil of two ages (20 and 40 years old). We calculated the relative
295 competition intensity (RCI) of both *P. purdomii* and *S. rehderiana* when subjected to different
296 planting patterns and soil ages, according to the formula described by Grace (1995) and Guo et al.
297 (2017) as follows: $RCI = (B_m - B_n)/B_n$, where B_m represents the dry biomass matter of one seedling
298 from interspecific planting and B_n represents the average dry biomass matter of corresponding

plants from intraspecific planting. If the RCI value is positive, the interspecific interaction has a facilitative effect on the species, while if the RCI value is negative, the interspecific interaction has a competitive effect on the species. Statistical analyses were conducted with the SPSS 16.0 for Windows statistical software package (SPSS, Chicago, IL, USA). Individual differences among treatments were compared by Tukey's tests following one-way ANOVAs. In addition, Generalized Linear Model was performed using the R statistical software (version 3.3.2) to evaluate the effects of species, soil age, planting pattern, and their interactions on each variable. All statistical tests were considered significant at $P < 0.05$. Redundancy analysis (RDA) was further undertaken to visualize the relationship between soil microbial community composition and soil properties (soil organic C, biochemical parameters, and enzyme activities), performed using Canoco 5.0 (Microcomputer Power, Ithaca, NY, USA).

3. Results

3.1. Effects of soil age and plant-plant interactions on morphological and physiological characteristics

In 20-year-old soil conditions, *S. rehderiana* individuals exhibited significantly higher aboveground dry matter (ADM), belowground dry matter (BDM) and total dry matter (TDM) weights when grown under interspecific interactions compared to intraspecific conditions (Fig. 2). However, in 40-year-old soil conditions, *P. purdomii* individuals showed significantly lower ADM, BDM and TDM under interspecific growth conditions when compared to intraspecific treatments (Fig. 2). On the other hand, *S. rehderiana* individuals from interspecific interactions showed lower ADM, BDM and TDM in 40-year-old soil compared to the values detected in 20-year-old soil. Furthermore, no significant differences were observed in the R/S ratio of *P. purdomii* and *S. rehderiana* between interspecific and intraspecific conditions in 20-year-old soil, whereas a significantly lower R/S ratio was detected in 40-year-old soil (Fig. 2d). In 20-year-old soil conditions, the relative competition intensity (RCI) value of *P. purdomii* and *S. rehderiana* was positive, which indicated that there was a cooperative relationship between the two species. However, in 40-year-old soil conditions, the RCI value of *P. purdomii* was negative, which indicated that *P. purdomii* was inhibited by competitive stress caused by *S. rehderiana* (Fig. 3). The growth traits of seedling (ADM, BDM and TDM) were significantly affected by species, soil age, species × soil age, species × planting pattern, and soil age × planting pattern (Table 4).

In 20-year-old soil conditions, *P. purdomii* individuals subjected to interspecific interactions showed significantly higher leaf and root starch contents than those exposed to intraspecific interactions, whereas *S. rehderiana* showed no significant differences in starch contents (Fig. 4a). *P. purdomii* individuals subjected to interspecific treatments had significantly higher leaf and root starch contents than those under intraspecific planting conditions, whereas *S. rehderiana* plants presented significantly lower leaf starch contents under interspecific planting than those subjected to intraspecific interactions when grown in 40-year-old soil (Fig. 4a). Under 20-year-old soil and interspecific growth conditions, *P. purdomii* individuals exhibited significantly higher root total sugar contents compared to those under intraspecific planting, whereas when grown in 40-year-old soil conditions, *P. purdomii* individuals growing under interspecific competition showed significantly lower root total sugar contents than those under intraspecific interactions (Fig. 4b). *P. purdomii* plants from intraspecific interactions exhibited significantly lower fructose and sucrose contents when grown in 40-year-old soil compared to those grown in 20-year-old soil (Fig. 4c and d). The interaction of species × soil age, species × planting pattern and species × soil age × planting pattern significantly affected leaf starch, while root starch was significantly affected by species, soil age, planting pattern, species × soil age and species × planting pattern (Table 4).

3.2. Effects of soil age and plant-plant interactions on nutrient absorption and allocation, and on foliage C and N isotopic composition

P. purdomii and *S. rehderiana* exhibited higher N and P contents but lower C:N ratios in leaves than in roots (Fig. 5). In 20-year-old soil conditions, *S. rehderiana* experiencing interspecific interactions

showed significantly higher leaf N contents than plants grown under intraspecific planting, whereas no significant differences in leaf N contents were observed in *P. purdomii*. The root N content of *P. purdomii* under interspecific planting was significantly higher in 40-year-old soil compared to that in 20-year-old soil. *P. purdomii* and *S. rehderiana* individuals exposed to interspecific interactions in 20-year-old soil showed higher root C:N ratios than those subjected to intraspecific conditions (Fig. 5c). *S. rehderiana* plants subjected to interspecific planting had higher leaf N:P ratios than those under intraspecific interactions in 20-year-old soil (Fig. 5d). Using a combination of 40-year-old soil and interspecific conditions, *S. rehderiana* plants exhibited lower root C:N ratios than those subjected to intraspecific treatment (Fig. 5c). The interaction of species × planting pattern, soil age × planting pattern, and species × soil age × planting pattern significantly affected leaf N contents and leaf C:N radio. Root N contents and root C:N ratio were significantly affected by soil age, planting pattern, species × soil age, and soil age × planting pattern (Table 4).

Under 20- and 40-year-old soil conditions, *P. purdomii* and *S. rehderiana* subjected to interspecific treatments had lower levels of $\delta^{15}N$ derived from NH_4^+ ($\delta^{15}N\text{-}NH_4^+$) than those growing under intraspecific conditions (Fig. 6a). *P. purdomii* and *S. rehderiana* exposed to interspecific treatments exhibited higher levels of $\delta^{15}N$ derived from NO_3^- ($\delta^{15}N\text{-}NO_3^-$) than plants grown under intraspecific conditions in 20-year-old soil (Fig. 6b). Under 40-year-old soil and interspecific conditions, the $\delta^{15}N\text{-}NO_3^-$ level of *S. rehderiana* significantly decreased compared to plants subjected to intraspecific interactions (Fig. 6b). In all treatments, *P. purdomii* individuals presented significantly higher $\delta^{13}C$ than *S. rehderiana*, whereas *S. rehderiana* individuals exposed to intra- and interspecific treatments exhibited no significant differences in $\delta^{13}C$. When using 40-year-old

soil, *P. purdomii* plants that experienced interspecific planting had significantly lower $\delta^{13}C$ than those exposed to intraspecific interactions (Fig. 6c).

3.3. Changes in leaf ultrastructure due to soil age and plant-plant interactions

When using 20-year-old soil, *P. purdomii* plants exposed to interspecific treatments showed smoother and thicker cell membranes and cell walls than those grown under intraspecific interactions (Fig. 8a and c). In addition, *S. rehderiana* plants that underwent interspecific treatments were characterized by fewer plastoglobuli and smaller starch granules than those subjected to intraspecific interactions (Fig. 8b and d). Under 40-year-old soil conditions, *P. purdomii* and *S. rehderiana* exposed to interspecific treatments presented negative plant-plant interactions, as indicated by fewer mitochondria, larger plastoglobuli and developing plasmolysis compared to intraspecific treatments (Fig. 8e-h). However, in 20-year-old soil conditions, *P. purdomii* and *S. rehderiana* grown under interspecific conditions were clearly influenced by positive plant-plant interactions, as indicated by typical chloroplast structures and well-arranged thylakoid membranes, and thick and continuous cytomembranes and cytoderms (Fig. 8a and b). In 40-year-old soil conditions, *P. purdomii* plants subjected to intraspecific treatments showed higher numbers of mitochondria with a normal structure and clear cristae, whereas *P. purdomii* plants exhibited more severe damage under interspecific planting patterns, as indicated by the disappearance of mitochondria, swollen chloroplasts and numerous plastoglobuli (Fig. 8e).

3.4. Response of soil biochemical properties and enzyme activities

Soil age significantly affected soil organic carbon (SOC), total nitrogen (TN), total potassium (TK), alkali-hydrolyzable nitrogen (AN), available potassium (AK), pH levels, and soil enzyme activities, whereas different planting patterns showed no differences in SOC, TN, total phosphorus (TP), and pH values (Table 1). *P. purdomii* and *S. rehderiana* individuals exposed to interspecific treatments showed higher soil AN contents than those grown under intraspecific interactions in 20-year-old soil. However, in 40-year-old soil conditions, interspecific *Populus*-*Salix* plantings showed lower soil TN contents compared to intraspecific conditions. Intra- and interspecific treatments using 40-year-old soil presented higher SOC and TN contents than those using 20-year-old soil. Thus, SOC and TN contents increased with aging soil. Small changes in soil enzyme activities were observed under different planting patterns in the same-age soil. In addition, *Populus*-*Salix* interactions resulted in significantly higher soil nitrate reductase activities compared to those of *Salix*-*Salix* interactions under 20-year-old soil conditions (Table 1). On the other hand, when using 40-year-old soil, *Populus*-*Salix* interactions exhibited significantly lower soil nitrate reductase activities. The activities of soil urease, invertase, and nitrate reductase were significantly higher in 40-year-old soil than in 20-year-old soil.

3.5. Soil microbial biomass and community structure characteristics

Under 20-year-old soil conditions, no significant differences were observed in MBC, MBN, or in the MBC:MBN ratio among the three planting patterns (interspecific *Populus*-*Salix*, intraspecific *Populus*-*Populus*, and intraspecific *Salix*-*Salix* planting). In 40-year-old soil conditions, interspecific

Populus-*Salix* planting resulted in lower MBC and MBN contents when compared to intraspecific *Salix*-*Salix* planting (Table 1). Intraspecific *Salix*-*Salix* treatments showed higher MBC and MBN contents in 40-year-old soil than in 20-year-old soil. Also, intraspecific *Populus*-*Populus* treatments presented higher MBC and MBN contents in 40-year-old soil than in 20-year-old soil. MBC and MBN contents were higher in 40-year-old soil compared to those in 20-year-old soil, and increasing soil microbial biomass affected plant-soil interactions as well (Table 1). The increase in soil microbial biomass is associated with a series of physicochemical processes between plant and soil. Under 20-year-old soil conditions, the *Populus*-*Salix* planting pattern showed no differences in soil microbes, whereas there were differences when 40-year-old soil was used.

No significant differences were observed in the total amounts of PLFAs among planting patterns in 20-year-old soil, whereas in 40-year-old soil, significant differences were observed (Table 2). Furthermore, under 40-year-old soil conditions, interspecific *Populus*-*Salix* planting exhibited lower densities of gram-positive bacteria, fungi (18:1ω9c), arbuscular mycorrhizal fungi (AMF), and actinomycetes, and lower total PLFA levels when compared to intraspecific planting. Under 20-year-old soil conditions, interspecific planting (PS) showed higher amounts of AMF and fungi (18:1ω9c) and higher ratios of gram+/gram- bacteria PLFAs and fungal/bacterial PLFAs when compared to intraspecific planting. However, in 40-year-old soil conditions, interspecific planting (PS) presented significantly lower amounts of AMF and fungi (18:1ω9c) and a lower ratio of fungal/bacterial PLFAs compared to intraspecific treatments (PP and SS). In 20-year-old soil under different planting patterns conditions, no significant changes were observed in the community structure of soil microbes, whereas under 40-year-old soil conditions, the effects of interspecific

453 *Populus-Salix* planting were significantly different from those detected in intraspecific treatments.
454 The community structures of soil microbes differed between 40-year-old soil and 20-year-old soil.
455
456 *3.6. Coupling relationship between soil microbes and abiotic factors*
457
458 The Pearson correlation analysis on soil microbial communities and abiotic factors indicated that
459 gram-positive bacteria (G+) and gram-negative bacteria (G-) were significantly correlated with soil
460 TN, alkali-hydrolyzable N, NH_4^+-N, and nitrate reductase activity ($P < 0.05$, Table 3). The total
461 amounts of PLFAs showed significant positive correlations with TN, NH_4^+-N, NO_3^--N, and nitrate
462 reductase activity. Soil actinomycetes showed significant positive correlations with SOC, TN,
463 NH_4^+-N, NO_3^--N, and enzyme activities. In addition, AM fungi exhibited significant positive
464 correlations with TN, NH_4^+-N, and NO_3^--N. Moreover, a significant positive correlation was
465 observed between fungi and alkali-hydrolyzable N contents. Therefore, the composition of soil
466 microbial communities was largely affected by soil nutrients (particularly N).
467
468 Redundancy analysis (RDA) revealed the effects of environmental factors on the composition of
469 soil microbial communities. The model explained 67.7% of PLFAs; RDA1 and RDA2 explained
470 53.1% and 14.6%, respectively, of the changes in the PLFA data (Fig. 7). RDA1 was positively
471 correlated with TN, AN, NH_4^+-N, NO_3^--N, and nitrate reductase activities. RDA2 was positively
472 correlated with SOC, pH, AP, urease, and saccharase. The analysis included SOC, TN, AN, TP, AP,
473 NH_4^+-N, NO_3^--N, pH, soil urease, saccharase, and nitrate reductase activities as environmental
474 factors. RDA showed that soil AN (which explained 36.8% of the variance, $P = 0.002$) was the most
475 important parameter contributing to the composition of soil microbial communities. Thereafter, the

most important ones were soil NH_4^+-N (which explained 33.2% of the variance, $P = 0.002$), soil TN (which explained 32.1% of the variance, $P = 0.002$), soil saccharase (which explained 29.5% of the variance, $P = 0.002$), SOC (which explained 28.3% of the variance, $P = 0.006$), soil NRA (which explained 28.2% of the variance, $P = 0.004$), soil urease (which explained 25.1% of the variance, $P = 0.01$), and soil NO_3^--N (which explained 24.1% of the variance, $P = 0.006$).

4. Discussion

Plant-plant interactions may induce negative and positive effects on subalpine forest communities. In fact, such negative and positive interactions occur widely in nature, particularly at high altitudes, thereby indicating that plant species are not independently distributed. The stress gradient hypothesis predicts that plant-plant interactions vary along external environmental gradients and that negative interactions are more frequent in relatively productive environments, whereas positive interactions are more common in severe environments (Bertness and Callaway, 1994; He et al., 2013). Indeed, many previous studies have shown that plant-plant interactions play a positive role in adaptation to severe environments in terms of survival, whereas neighboring plants undergo competition in low-stress environments to avoid elimination by other species (Callaway and Walker, 1997; Fynn et al., 2005; Craine and Dybzinski, 2013). Contrary to facilitative interactions, competitive interactions between neighboring species induce negative interference (Bertness and Callaway, 1994; Brooker and Callaghan, 1998).

The present study demonstrated that positive and negative interactions affect the growth traits (biomass allocation, nutrient absorption, mesophyll cells) of *P. purdomii* and *S. rehderiana* individuals in 20- and 40-year-old soil. Furthermore, our data suggested that when soil nutrient resources are limited (e.g., 20-year-old soil), *Populus-Salix* interactions result in higher above- and belowground dry matter accumulation, foliage starch contents, root C/N ratios and $\delta^{15}N\text{-}NO_3^-$ values compared with intraspecific conditions. Thus, there are positive interactions between *P. purdomii* and *S. rehderiana* individuals when grown in 20-year-old soil. Previous research has

showed that neighboring plants could improve nutrient availability and micro-climate, which would then ameliorate plant performance under high abiotic stress (Maestre et al., 2005). Conversely, when soil nutrient resources are moderate (e.g., 40-year-old soil), *P. purdomii* and *S. rehderiana* subjected to interspecific interactions show a lower rate of dry matter accumulation and lower root/shoot ratios, thus indicating that root growth is inhibited by interspecific competition and there is a negative interaction. In addition, mesophyll cells of *P. purdomii* plants showed tissue damage under interspecific conditions when grown in 40-year-old soil, as indicated by the disappearance of mitochondria, numerous plastoglobuli and developing plasmolysis. As also previously shown, the effect of neighbors is facilitation under low abiotic stress and competition under high stress (Maestre et al., 2005). For instance, Kikvidze et al. (2006) found that plant-plant interactions switched from competition to facilitation when the stress intensity increased (water limitation) in subalpine plant communities. Moreover, Olsen et al. (2016) showed a temperature-driven switch in plant-plant interactions from facilitation to competition in seminatural grasslands. Our study, which suggests that changes in the level of soil nutrients mediate *Populus-Salix* interactions from positive to negative in the subalpine glacier retreat areas, provides further support for the results of Brooker and Callaghan (1998) indicating that the intensity of positive interactions increases and that of negative interactions decreases with increasing stress and disturbance.

Plant-soil feedbacks influence plant performance (leaf and root traits) and competitive ability (Kardol et al., 2007; Teste et al., 2017), which could regulate nutrient cycling in ecosystems (Bohlen et al., 2001; Mariotte et al., 2018). Plant growth could change the biochemical properties of soil in a way that affects plant-plant interactions. Our results showed that soil alkali-hydrolyzable N and

542 NO_3^--N contents decrease under interspecific treatment (PS) compared to intraspecific treatments
543 (SS and PP) in 40-year-old soil, whereas in 20-year-old soil, alkali-hydrolyzable N and NO_3^--N of
544 PS increase compared to those in SS and PP treatments (Table 1). Soil N gradually accumulates
545 through primary succession, which directly affects the composition of microbial communities and
546 indirectly regulates plant-plant interactions in a glacier forefield. Consequently, plant-soil feedbacks
547 lead to the replacement of early-successional species by late-successional species, as
548 early-successional species suffer from negative plant-soil feedbacks (Kulmatiski et al., 2008).
549 Moreover, our results demonstrated that SOC, NH_4^+-N and NO_3^--N contents were significantly
550 higher in 40-year-old soil than in 20-year-old soil, thereby indicating that soil C and N contents
551 significantly accumulate in the studied glacier forefield over time. In addition, significantly higher
552 soil enzyme activities were observed in older soil, whereas no differences in enzyme activities in
553 relation to intra- and interspecific interactions were observed, thus indicating that the effects of
554 plant-plant interactions on enzyme activities were relatively small (Table 1). Biochemical properties
555 and extracellular enzyme activities are considered as important indicators of soil fertility
556 (Vepsäläinen et al., 2001; Mairura et al., 2007).
557
558 Soil microbes play an important role in the soil development process of primary succession,
559 particularly in glacier retreat regions. Microbes affect plant growth, and feedbacks occur because
560 plants alter microbial communities through root exudates (Westover et al., 1997; Stephan et al.,
561 2000). Wardle et al. (2004) have shown that the decomposer subsystem breaks down dead plant
562 material that indirectly regulates plant growth, and plant litter, in turn, provides energy (C) and
563 nutrients that are required by soil microbes. Moreover, Kardol et al. (2007) have reported that

564 early-successional plants generally change the composition of soil microbial communities and
565 increase the likelihood for the establishment of mid-successional species. Our findings suggest that
566 under 20-year-old soil conditions, there are no significant differences in the soil microbial biomass
567 and community composition between intra- and interspecific treatments of *P. purdomii* and *S.*
568 *rehderiana*. However, under 40-year-old soil conditions, interspecific planting of *Populus-Salix*
569 shows significantly lower amounts of gram-negative bacteria, gram-positive bacteria, AMF, fungi,
570 actinomycetes, and total PLFAs in soil when compared to intraspecific conditions (Table 2). These
571 findings suggest that the composition of soil microbial communities under interspecific conditions
572 substantially differs from that under intraspecific treatments and that belowground competitive
573 interactions decrease soil microbial biomass and change microbial community structures.
574
575 The aboveground and belowground linkages have a widely influence on ecosystem processes. The
576 aboveground biota can influence belowground subsystems. As a feedback, belowground organisms
577 could influence plant growth and the structure and function of the aboveground community (Wardle
578 et al., 2004). Plant-soil feedbacks are mechanisms of plant-plant interactions that are dependent on
579 competition for resources and changes in soil properties. Bever et al. (1997) have demonstrated that
580 soil microbes could alter the coexistence of plant species through indirect feedbacks. Furthermore,
581 the composition of soil microbial communities has been shown to have a strong effect on plant-plant
582 interactions, community dynamics and coexistence (Van der Putten and Peters, 1997; Van Der
583 Heijden et al., 2006). Our findings indicate that microbial community compositions (e.g., the
584 amount of PLFAs) have significant correlations with soil TN, AN, NH_4^+-N, NO_3^--N and nitrate
585 reductase activity (Table 3, $P < 0.05$). These results suggest that nitrogen availability in soil is

586 essential for microbial growth, and it directly affects the growth of plants and the interactions of
587 neighboring plants.
588
589 Soil microbial effects are incorporated into the dynamic changes of plant communities through
590 niche modification and plant-soil feedbacks (Kardol et al., 2007; Bever et al., 2010). Plant-plant
591 interactions could be directly driven by sharing of resources or competition, and the interactions of
592 plant and soil microbes could mediate the N transformation process of soil (Bohlen et al., 2001).
593 Göransson et al. (2011) have shown that the availability of resources and nutrient limitation for
594 microbial growth vary along a chronosequence in a glacier forefield, thereby suggesting that
595 microbial growth is mainly limited by C and plant growth is mainly limited by N, whereas soil
596 microbes are important competitors for N in young soil. Our results show that soil N availability
597 (NH_4^+ and NO_3^-) are the most important parameters that contribute to the composition of soil
598 microbial communities (Fig. 7). Furthermore, Reynolds et al. (2003) have demonstrated that
599 microbe-mediated partitioning of soil resources could contribute to the coexistence of plant species
600 through associations with different microbial symbionts. Hence, soil microbes alter the availability
601 of different forms of N, and indirectly affect plant-plant interactions by mediating soil resource
602 partitioning.

5. Conclusions

The present study shows that there are soil age-driven changes in *Populus-Salix* interactions, from positive to negative during the primary succession in the Gongga Mountain glacier retreat area. Our study reveals that *Populus-Salix* interactions exhibit facilitation for survival under 20-year-old soil, whereas under 40-year-old soil conditions, *Populus-Salix* interactions involve competition to avoid elimination. Moreover, soil microbes and nutrients (particularly N) are major factors for the transformation of *Populus-Salix* interactions from positive to negative. Our results support the stress gradient hypothesis, which predicts that positive and negative interactions vary inversely along abiotic stress gradients, with positive interaction being more common under high abiotic stress when compared to more benign abiotic conditions. These findings improve our understanding of plant-plant interactions and plant-soil feedbacks in subalpine forest ecosystems. However, further research is still needed on energy flows and nutrient cycles in plant-soil-microbe systems, on the effects of neighboring plants and microbial feedbacks, and on the dynamic models of primary succession in a glacier retreat area.

Acknowledgements This work was supported by the Talent Program of the Hangzhou Normal University (2016QDL020). We thank LetPub (www.letpub.com) for its linguistic assistance during the preparation of this manuscript.

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Table 1. Changes of soil chemical properties, extracellular enzyme activities, soil microbial biomass C, and microbial biomass N under different soil age and plant-plant interaction conditions.

Soil properties	20-year-old soil			40-year-old soil		
	PP	SS	PS	PP	SS	PS
SOC ($g \cdot kg^{-1}$)	6.52 ± 0.24 b	7.57 ± 0.31 b	6.98 ± 0.11 b	16.75 ± 0.41 a	17.47 ± 0.89 a	16.38 ± 0.19 a
TN ($g \cdot kg^{-1}$)	0.49 ± 0.03 b	0.56 ± 0.02 b	0.56 ± 0.04 b	0.74 ± 0.03 a	0.81 ± 0.06 a	0.73 ± 0.02 a
TP ($g \cdot kg^{-1}$)	1.30 ± 0.05 a	1.23 ± 0.02 a	1.26 ± 0.05a	1.18 ± 0.10 a	1.54 ± 0.19 a	1.17 ± 0.07 a
TK ($g \cdot kg^{-1}$)	48.77 ± 0.32 ab	48.77 ± 0.17 ab	50.02 ± 0.26 a	48.12 ± 0.80 abc	45.97 ± 0.23 c	46.97 ± 0.78 bc
AN ($mg \cdot kg^{-1}$)	93.30 ± 2.77 c	95.15 ± 3.00 c	102.68 ± 5.58 c	143.98 ± 6.16 ab	154.21 ± 3.73 a	129.77 ± 3.91 b
AP ($mg \cdot kg^{-1}$)	60.79 ± 0.80 ab	54.76 ± 1.27 bc	61.17 ± 1.83 ab	53.46 ± 1.49 c	62.54 ± 0.59 a	60.25 ± 2.48 ab
AK ($mg \cdot kg^{-1}$)	187.04 ± 1.85 ab	183.47 ± 3.74 b	207.64 ± 5.89 a	181.17 ± 4.98 b	165.38 ± 1.94 b	183.63 ± 7.06 b
NH_4^+-N ($mg \cdot kg^{-1}$)	15.47 ± 0.93 b	15.76 ± 0.42 b	15.63 ± 1.37 b	21.04 ± 1.07 a	22.51 ± 1.39 a	19.63 ± 0.79 a
NO_3^--N ($mg \cdot kg^{-1}$)	33.68 ± 2.22 b	31.44 ± 1.35 b	33.53 ± 2.94 b	42.38 ± 1.47 a	44.16 ± 1.85 a	40.58 ± 3.19 a
MBC ($mg \cdot kg^{-1}$)	256.26 ± 4.67 c	274.11 ± 3.25 c	246.69 ± 5.44 cd	345.40 ± 5.98 ab	347.05 ± 4.96 a	323.76 ± 3.51 b
MBN ($mg \cdot kg^{-1}$)	9.24 ± 0.25 c	9.51 ± 0.14 c	8.90 ± 0.19 c	15.49 ± 0.38 ab	16.55 ± 0.35 a	14.53 ± 0.13 b
MBC:MBN ratio	27.76 ± 0.96 a	28.83 ± 0.49 a	27.72 ± 0.14 a	22.33 ± 0.73 b	20.98 ± 0.22 b	22.29 ± 0.34 b
Urease activity ($\mu g\ NH_4\text{-}N \cdot g^{-1} \cdot soil \cdot h^{-1}$)	15.19 ± 0.39 b	13.81 ± 0.28 b	13.17 ± 0.47 b	19.23 ± 0.47 a	19.94 ± 0.41 a	19.52 ± 0.81 a
Nitrate reductase activity ($\mu g\ NO_2\text{-}N \cdot g^{-1} \cdot soil \cdot h^{-1}$)	5.39 ± 0.25 bc	4.06 ± 0.51 b	4.83 ± 0.33 bc	6.73 ± 0.57 ab	7.68 ± 0.17 a	6.47 ± 0.57 ab
Saccharase activity ($\mu g\ glucose \cdot g^{-1} \cdot soil \cdot h^{-1}$)	132.79 ± 3.21 b	139.46 ± 3.38 b	126.98 ± 1.86 b	184.81 ± 2.92 a	180.71 ± 4.09 a	175.89 ± 4.06 a
pH value	6.65 ± 0.05 a	6.80 ± 0.10 a	6.68 ± 0.05 a	6.03 ± 0.15 b	6.52 ± 0.19 ab	6.00 ± 0.11 b

SOC, soil organic carbon; TN, soil total nitrogen; TP, soil total phosphorus; TK, soil total potassium; AN, soil alkali-hydrolyzable nitrogen; AP, soil available phosphorus; AK, soil available potassium; NH_4^+-N, soil ammonium nitrogen; NO_3^--N, soil nitrate nitrogen; MBC, soil microbial biomass C; MBN, soil microbial biomass N. PP, soil sample from *P. purdomii* and *P. purdomii* intraspecific interaction; SS, soil sample from *S. rehderiana* and *S. rehderiana* intraspecific interaction; PS, soil sample from *P. purdomii* and *S. rehderiana* interspecific interaction. Values followed by same letters in the same row are not significantly different at the $P < 0.05$ level according to Tukey's test. Values are expressed as means ± SE ($n = 4$).

873 **Table 2.** Changes of soil microbial PLFA parameters (nmol g^{-1} soil) under difference soil age and plant-plant
874 interactions conditions.

PLFA markers	20-year-old soil			40-year-old soil		
	PP	SS	PS	PP	SS	PS
Gram-positive						
i14:0	0.036±0.003 a	0.034±0.003 a	0.022±0.005 a	0.036±0.008 a	0.049±0.007 a	0.036±0.004 a
i15:0	0.497±0.039 a	0.503±0.033 a	0.533±0.035 a	0.534±0.121 a	0.627±0.056 a	0.405±0.010 a
i15:1 G	0.083±0.009 a	0.082±0.007 ab	0.028±0.016 b	0.090±0.015 a	0.093±0.013 a	0.071±0.005 ab
i16:0	0.147±0.004 b	0.171±0.006 ab	0.164±0.003 b	0.204±0.012 a	0.205±0.007 a	0.151±0.007 b
i17:0	0.156±0.006 ab	0.143±0.010 ab	0.137±0.008 ab	0.174±0.015 ab	0.205±0.029 a	0.115±0.009 b
a15:0	0.329±0.024 a	0.337±0.015 a	0.361±0.038 a	0.406±0.077 a	0.466±0.033 a	0.303±0.013 a
a17:0	0.145±0.003 ab	0.167±0.006 ab	0.093±0.017 b	0.190±0.013 ab	0.206±0.023 a	0.126±0.005 ab
Σ Gram-positive	1.392±0.076 ab	1.437±0.054 ab	1.339±0.008 ab	1.634±0.244 ab	1.851±0.169 a	1.207±0.033 b
Gram-negative						
17:1 ω8c	0.060±0.003 a	0.085±0.011 a	nd	0.063±0.005 a	0.072±0.018 a	0.048±0.004 a
18:1 ω5c	0.126±0.011 a	nd	nd	0.164±0.022 a	0.139±0.008 a	0.126±0.012 a
cy17:0	0.333±0.005 bc	0.400±0.005 ab	0.360±0.016 bc	0.377±0.017 abc	0.468±0.038 a	0.285±0.017 c
cy19:0 ω8c	0.362±0.004 a	0.363±0.018 a	0.369±0.009 a	0.359±0.032 a	0.439±0.016 a	0.185±0.018 b
16:1 2OH	nd	0.041±0.005 b	nd	0.028±0.005 bc	0.063±0.007 a	0.015±0.001 cd
Σ Gram-negative	0.882±0.018 bc	0.889±0.054 bc	0.729±0.036 c	0.991±0.145 ab	1.181±0.151 a	0.659±0.050 c
AM fungi						
16:1 ω5c	0.245±0.003 c	0.264±0.003 bc	0.341±0.043 abc	0.364±0.034 ab	0.400±0.024 a	0.233±0.008 c
16:1 ω11c	0.115±0.005 b	0.125±0.005 ab	0.132±0.008 ab	0.163±0.022 ab	0.176±0.017 a	0.106±0.005 b
Σ AMF	0.360±0.003 c	0.389±0.005 bc	0.473±0.042 abc	0.527±0.036 ab	0.576±0.032 a	0.340±0.012 c
Fungi						
18:1 ω9c	0.565±0.024 bc	0.533±0.031 bc	0.571±0.012 bc	0.669±0.053 ab	0.768±0.065 a	0.390±0.021 c
Actinomycetes						
10 Me17:0	0.021±0.002 a	0.021±0.001 a	0.023±0.002 a	0.039±0.009 a	0.039±0.008 a	0.021±0.005 a
10 Me18:0	0.136±0.004 b	0.148±0.012 b	0.108±0.011 b	0.262±0.023 a	0.228±0.024 a	0.122±0.002 b
ΣActinomycetes	0.157±0.002 b	0.169±0.012 b	0.131±0.009 b	0.301±0.029 a	0.267±0.032 a	0.144±0.005 b
Total PLFAs	3.356±0.162 bc	3.418±0.211 bc	3.244±0.122 bc	4.122±0.765 ab	4.644±0.639 a	2.739±0.109 c
Gram+/Gram- ratio	1.576±0.098 a	1.617±0.094 a	1.842±0.127 a	1.632±0.180 a	1.563±0.051 a	1.839±0.155 a
Fungi/Bacteria ratio	0.249±0.028 abc	0.229±0.012 bc	0.276±0.005 a	0.258±0.019 ab	0.253±0.009 abc	0.209±0.015 c

875 PP, soil sample from *P. purdomii* and *P. purdomii* intraspecific interaction; SS, soil sample from *S. rehderiana* and
876 *S. rehderiana* intraspecific interaction; PS, soil sample from *P. purdomii* and *S. rehderiana* interspecific interaction.
877 Values followed by different letters in the same row are significantly different at the $P < 0.05$ level according to
878 Tukey's test. nd, not detected. Values are expressed as means ± SE ($n = 4$).

879 **Table 3.** Pearson correlation coefficients between soil biochemical properties and microbial lipid biomarkers.

	SOC	TN	AN	pH	NH_4^+-N	NO_3^--N	Urease	Saccharase	Nitrate reductase
G+	0.392	**0.549***	**0.559***	0.058	**0.525***	0.415	0.346	0.407	**0.507***
G-	0.368	**0.481***	**0.529***	0.078	**0.508***	0.402	0.382	0.399	**0.521***
G+/G- ratio	-0.016	0.028	-0.059	-0.082	-0.103	-0.058	-0.175	-0.077	-0.172
Bacteria	0.391	**0.533***	**0.559***	0.068	**0.529***	0.418	0.368	0.413	**0.524***
Fungi	0.275	**0.473***	**0.471***	0.096	0.438	0.377	0.232	0.276	0.433
F/B ratio	-0.211	-0.104	-0.070	0.158	-0.081	-0.023	-0.249	-0.246	-0.069
AM Fungi	0.426	**0.609****	**0.644****	-0.066	**0.597***	**0.528***	0.333	0.416	**0.515****
Actinomycetes	**0.650****	**0.690****	**0.746*****	-0.346	**0.669****	**0.598****	**0.582***	**0.707****	**0.615****
Total PLFAs	0.419	**0.568***	**0.601****	0.017	**0.562***	**0.475***	0.378	0.438	**0.541***

880 G+, gram-positive bacterial PLFAs; G-, gram-negative bacterial PLFAs; G+/G-, the ratio of gram-positive to
881 gram-negative bacterial PLFAs; F/B, the ratio of fungal (18:1 ω9c) to bacterial PLFAs; AM Fungi, arbuscular
882 mycorrhizal fungi; SOC, soil organic carbon; TN, soil total nitrogen; AN, alkali-hydrolyzable nitrogen. Significant
883 correlations are shown in bold. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

884 **Table 4.** Generalized Linear Model (GLM) results of species, soil age, plant pattern, and their interactions effects
885 on morphological traits, nutrient allocation and non-structural carbohydrates. F_S, species effect; F_Y, soil age effect;
886 F_p, planting pattern effect; $F_{S\times Y}$, species × soil age effect; $F_{S\times P}$, species × planting pattern effect; $F_{Y\times P}$, soil age ×
887 planting pattern effect; $F_{S\times Y\times P}$, species × soil age × planting pattern effect. Significant effects ($P < 0.05$) are shown
888 in a bold font.

Variable	$P>F_S$	$P>F_Y$	$P>F_P$	$P>F_{S\times Y}$	$P>F_{S\times P}$	$P>F_{Y\times P}$	$P>F_{S\times Y\times P}$
Aboveground	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.203
Belowground	**<0.001**	**<0.001**	0.055	**0.002**	**<0.001**	**<0.001**	0.444
Total biomass	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.099
Root:shoot radio	**<0.001**	**0.033**	**0.025**	0.126	0.868	**0.002**	0.868
Leaf N	**0.019**	**<0.001**	0.641	0.051	**<0.001**	**0.009**	**<0.001**
Leaf P	0.322	0.098	0.953	0.269	0.150	0.860	0.861
Leaf C:N	**0.002**	**<0.001**	0.099	0.118	**0.007**	**0.015**	**0.013**
Leaf N:P	0.472	**<0.001**	0.811	**0.023**	**<0.001**	0.052	**0.012**
Root N	0.927	**<0.001**	**<0.001**	**<0.001**	0.116	**<0.001**	0.415
Root P	**0.015**	**0.015**	**0.002**	0.166	0.351	0.352	0.814
Root C:N	0.307	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.298
Root N:P	**<0.001**	0.883	0.401	**<0.001**	**0.011**	0.089	0.304
Leaf starch	0.333	0.866	**0.004**	**<0.001**	**<0.001**	0.275	**<0.001**
Leaf total sugar	**<0.001**	**<0.001**	0.595	**<0.001**	0.149	0.814	0.072
Leaf sucrose	**<0.001**	**<0.001**	**<0.001**	0.912	0.407	0.231	**0.013**
Leaf fructose	**<0.001**	**<0.001**	**0.013**	0.618	0.266	0.192	0.197
Root starch	**<0.001**	**0.002**	**0.039**	**0.001**	**<0.001**	0.772	0.295
Root total sugar	**<0.001**	**<0.001**	0.269	**<0.001**	0.538	**<0.001**	**0.031**
Root sucrose	**<0.001**	**0.002**	0.408	0.406	0.051	0.363	0.442
Root fructose	**<0.001**	**<0.001**	0.134	0.515	**0.006**	0.468	0.093

889

Figure legends

Figure 1. The experiment was conducted on the Gongga Mountain glacier forefield, which is located on the south-eastern fringe of the Tibetan Plateau (modified from Zhou et al. 2013). A, experimental station; S3, 20-year-old soil; S4, 40-year-old soil. S1-S7 belong to a 120-year-old soil chronosequence.

Figure 2. Growth traits of *P. purdomii* and *S. rehderiana* as affected by different soil age and plant-plant interaction conditions. Different letters above bars denote statistically significant differences between treatments at the $P < 0.05$ level according to Tukey's test. Generalized Linear Model was conducted to evaluate the effects of different factors and their interactions (Table 4). Treatment codes are as follows: P/PP, *P. purdomii* individuals from intraspecific interaction; P/PS, *P. purdomii* individuals from interspecific interaction; S/SS, *S. rehderiana* individuals from intraspecific interaction; S/PS, *S. rehderiana* individuals from interspecific interaction. Values are expressed as means ± SE ($n = 4$).

Figure 3. Relative competition intensity of *P. purdomii* and *S. rehderiana* under different soil age and plant-plant interaction conditions. Different letters above bars denote statistically significant differences between treatments at the $P < 0.05$ level according to Tukey's test.

Figure 4. Leaf and root starch (a), total sugar (b), sucrose (c) and fructose (d) contents in *P. purdomii* and *S. rehderiana* plants under different soil age and plant-plant interaction conditions. Treatment codes are the same as those in Figure 2. Different letters above bars denote statistically significant differences between treatments at the $P < 0.05$ level according to Tukey's test. *P* values for interactive effects according to Generalized Linear Model was in Table 4. Values are expressed as means ± SE ($n = 4$).

Figure 5. Leaf and root nitrogen (a), phosphorus (b), the ratio of carbon to nitrogen (c) and the ratio of nitrogen to phosphorus (d) contents in *P. purdomii* and *S. rehderiana* plants under different soil age and plant-plant interaction conditions. Treatment codes are the same as those in Figure 2. Different letters above bars denote statistically significant differences between treatments at the $P < 0.05$ level according to Tukey's test. *P* values for interactive effects according to Generalized Linear Model was in Table 4. Values are expressed as means ± SE ($n = 4$).

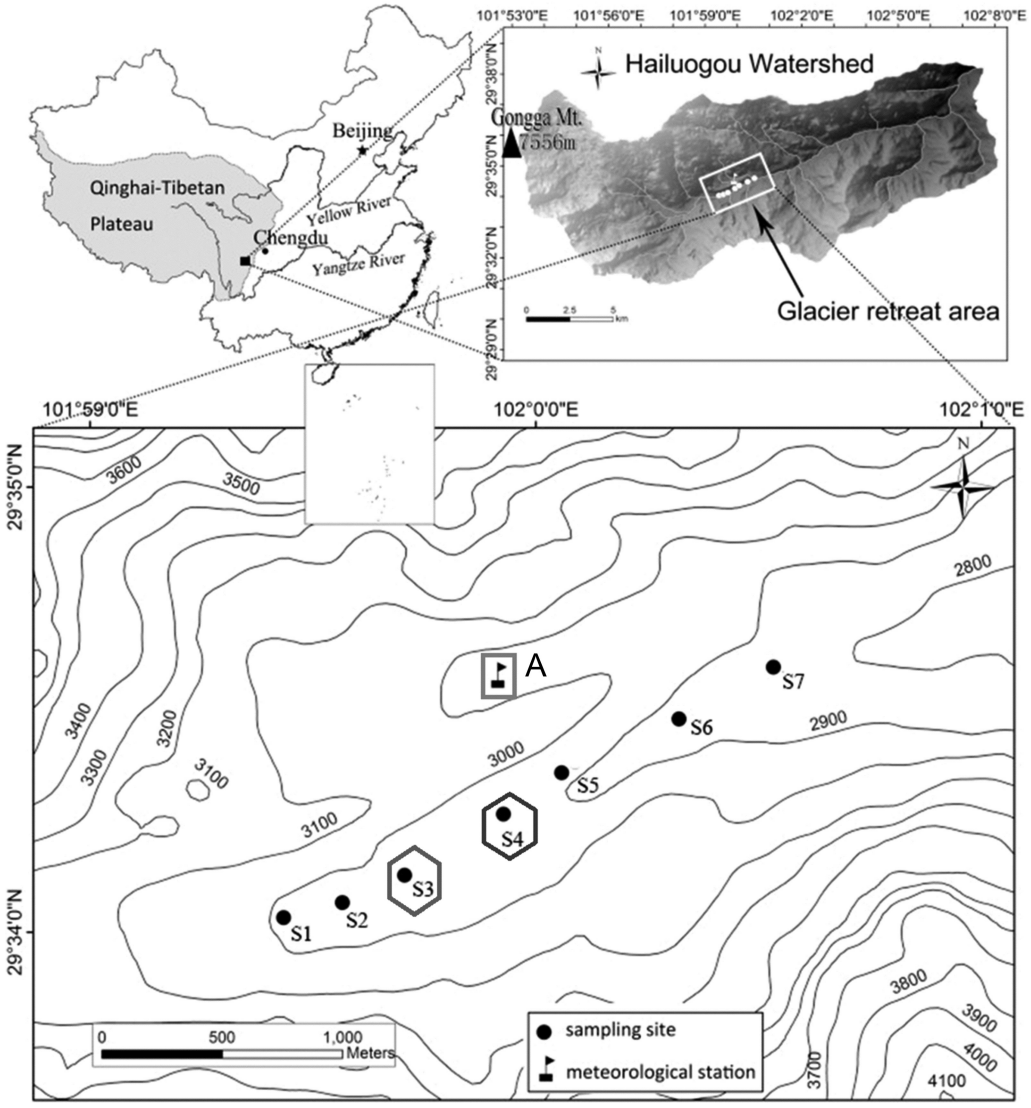
Figure 6. Foliage $\delta^{15}N$ derived from NH_4^+ ($\delta^{15}N\text{-}NH_4^+$), $\delta^{15}N$ derived from NO_3^- ($\delta^{15}N\text{-}NO_3^-$) and carbon isotope

composition ($\delta^{13}C$) of *P. purdomii* and *S. rehderiana* under different soil age and plant-plant interaction conditions. Treatment codes are the same as those in Figure 2. Different letters above bars denote statistically significant differences between treatments at the $P < 0.05$ level according to Tukey's test. Values are expressed as means ± SE ($n = 4$).

Figure 7. Redundancy analysis (RDA) between soil microbial PLFAs and environmental parameters. White square, soil sample from interspecific interaction of *P. purdomii* and *S. rehderiana* in 20-year-old soil; white star, soil sample from intraspecific interaction of *P. purdomii* and *P. purdomii* in 20-year-old soil; white inverse triangle, soil sample from intraspecific interaction of *S. rehderiana* and *S. rehderiana* in 20-year-old soil; black square, soil sample from interspecific interaction of *P. purdomii* and *S. rehderiana* in 40-year-old soil; black star, soil sample from intraspecific interaction of *P. purdomii* and *P. purdomii* in 40-year-old soil; black inverse triangle, soil sample from intraspecific interaction of *S. rehderiana* and *S. rehderiana* in 40-year-old soil; G+, gram-positive bacteria PLFAs; G-, gram-negative bacteria PLFAs; G+/G-, the ratio of gram-positive bacteria to gram-negative bacteria PLFAs; F/B, the ratio of fungi (18:1 ω9c) to bacteria PLFAs; AM Fungi, arbuscular mycorrhizal fungi; MBC, soil microbial biomass C; MBN, soil microbial biomass N; SOC, soil organic carbon; TN, soil total nitrogen; TP, soil total phosphorus; AN, soil alkali-hydrolyzable nitrogen; AP, soil available phosphorus; NH_4^+-N, soil ammonium nitrogen; NO_3^--N, soil nitrate nitrogen.

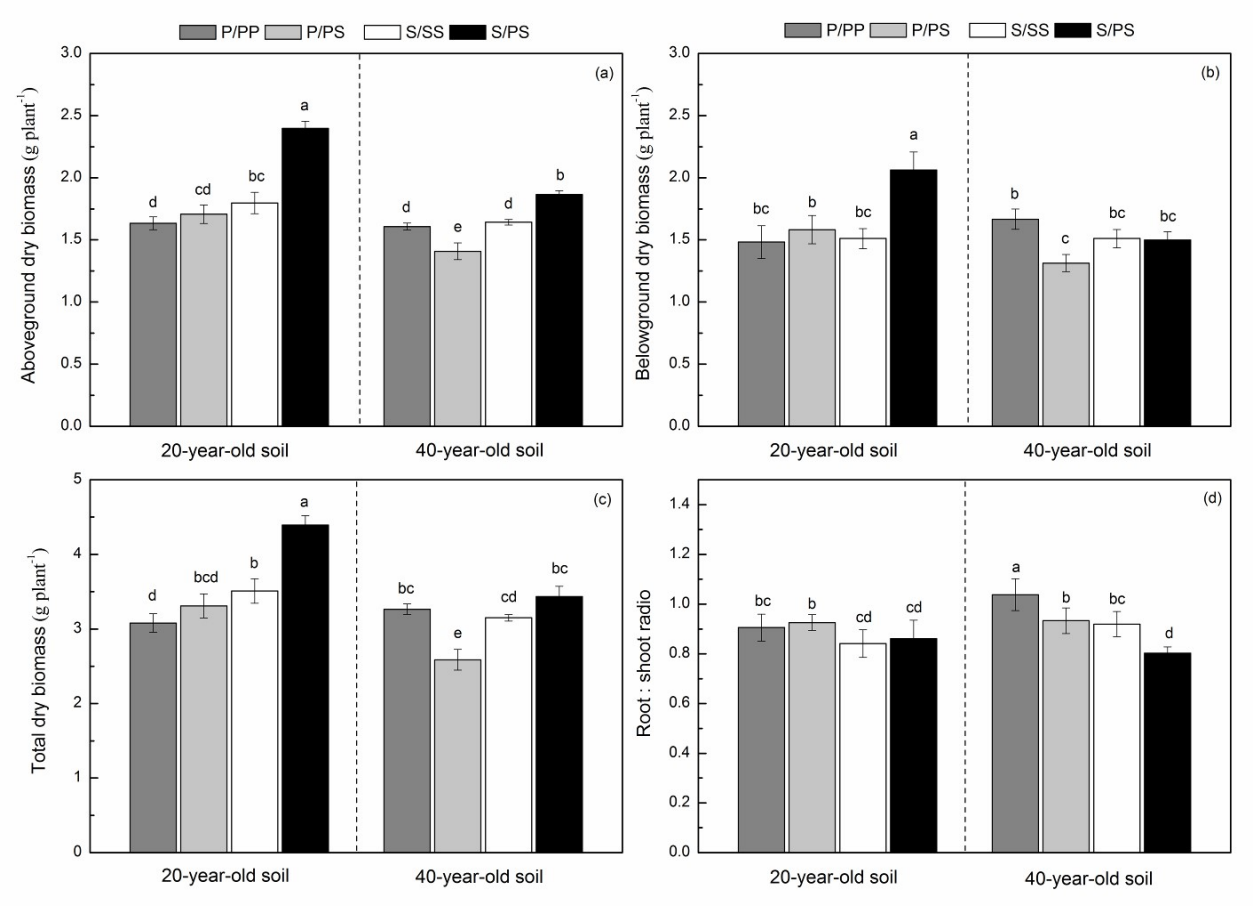
Figure 8. Transmission electron microscopy observations of mesophyll cells in *P. purdomii* and *S. rehderiana* under different soil age and planting pattern conditions. (a) *P. purdomii* from interspecific interaction in 20-year-old soil; (b) *S. rehderiana* from interspecific interaction in 20-year-old soil; (c) *P. purdomii* from intraspecific interaction in 20-year-old soil; (d) *S. rehderiana* from intraspecific interaction in 20-year-old soil; (e) *P. purdomii* from interspecific interaction in 40-year-old soil; (f) *S. rehderiana* from interspecific interaction in 40-year-old soil age; (g) *P. purdomii* from intraspecific interaction in 40-year-old soil; (h) *S. rehderiana* from intraspecific interaction in 40-year-old soil. C, chloroplast; S, starch granule; P, plastoglobulus; G, granum; CW, cell wall; V, vacuole; M, mitochondrion; N, nucleus. The bars correspond to 1 μm in all panels.

946 **Figure 1.**

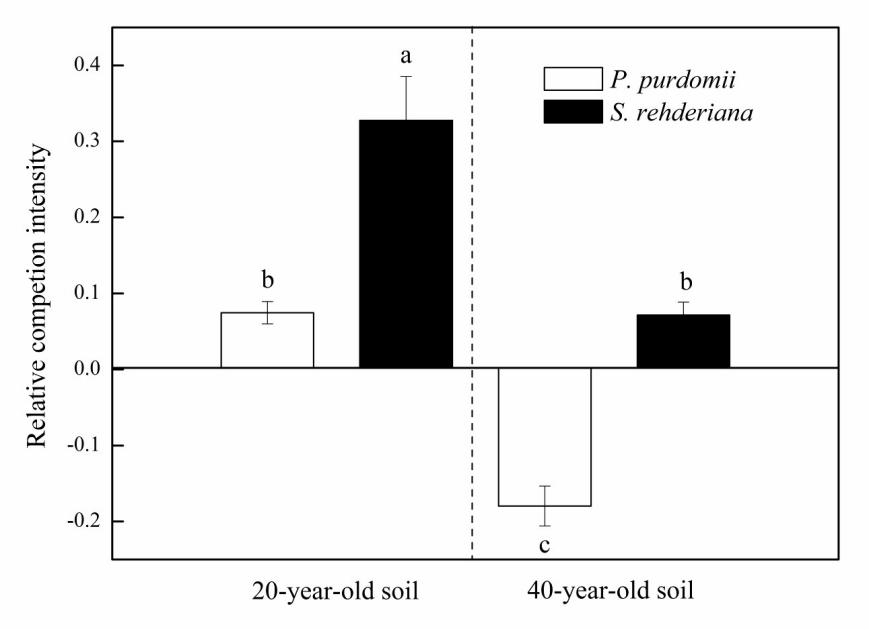


947

Figure 2.


P/PP
P/PS
S/SS
S/PS
(a)
Aboveground dry biomass (g plant-1)
(b)
Belowground dry biomass (g plant-1)
(c)
Total dry biomass (g plant-1)
(d)
Root : shoot radio
20-year-old soil
40-year-old soil

951 **Figure 3.**



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953

Figure 4.

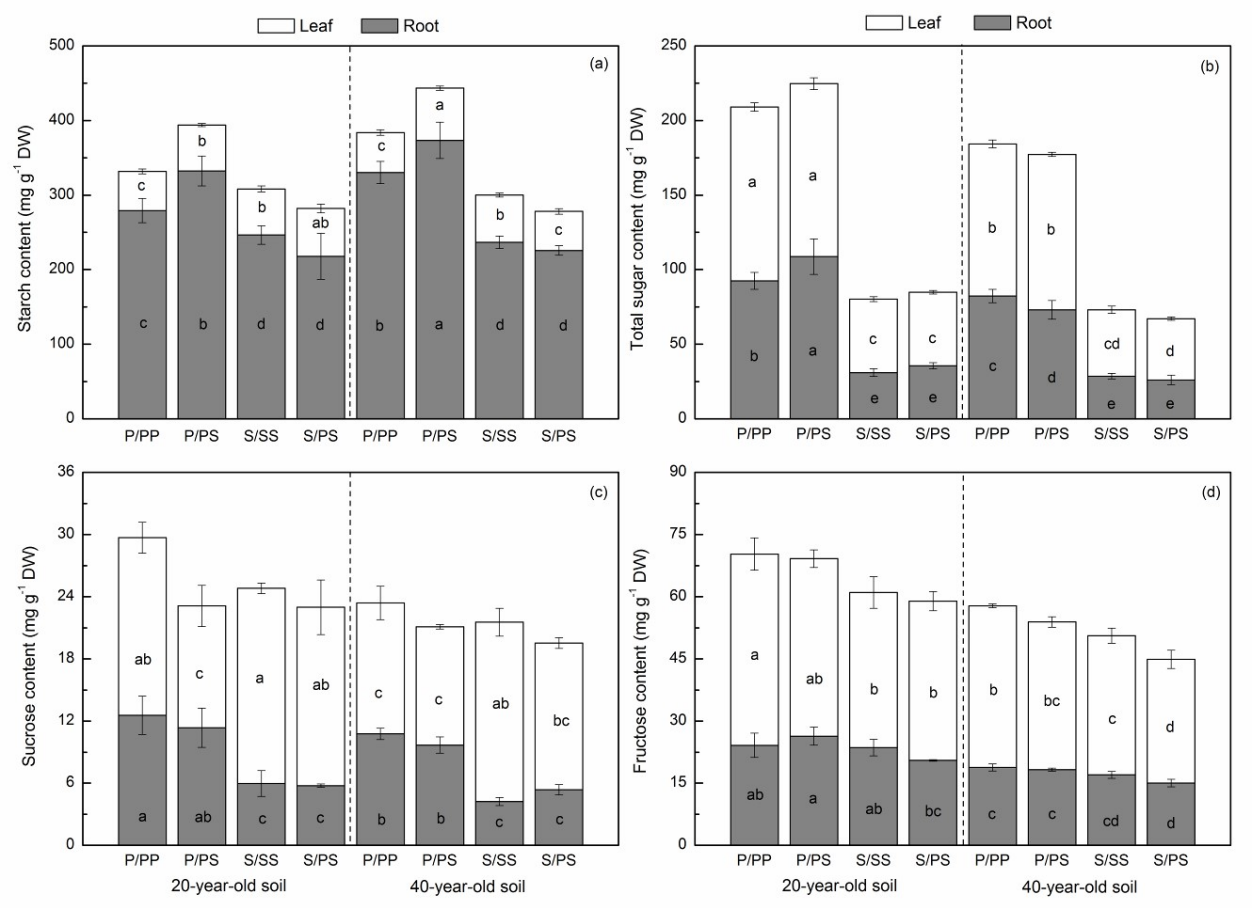
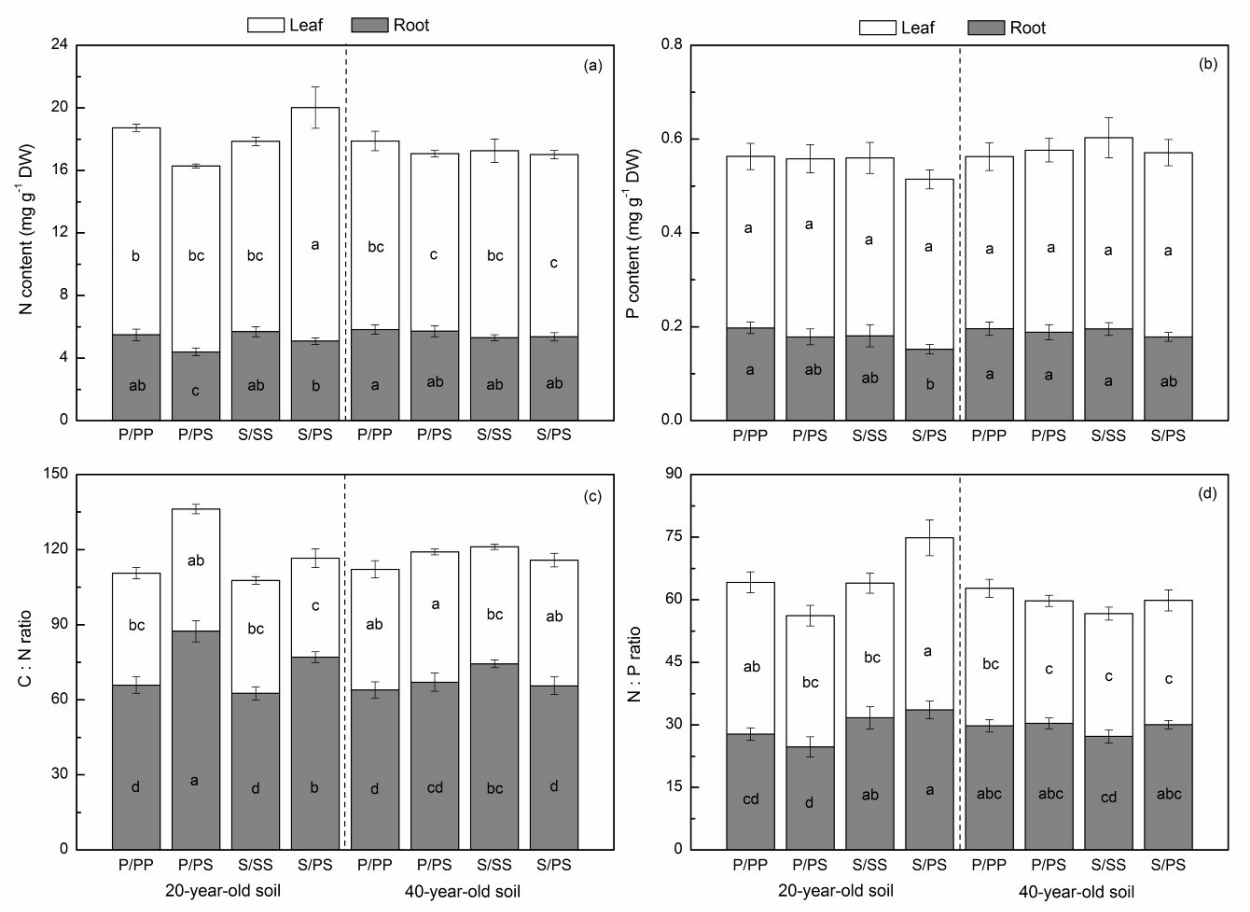
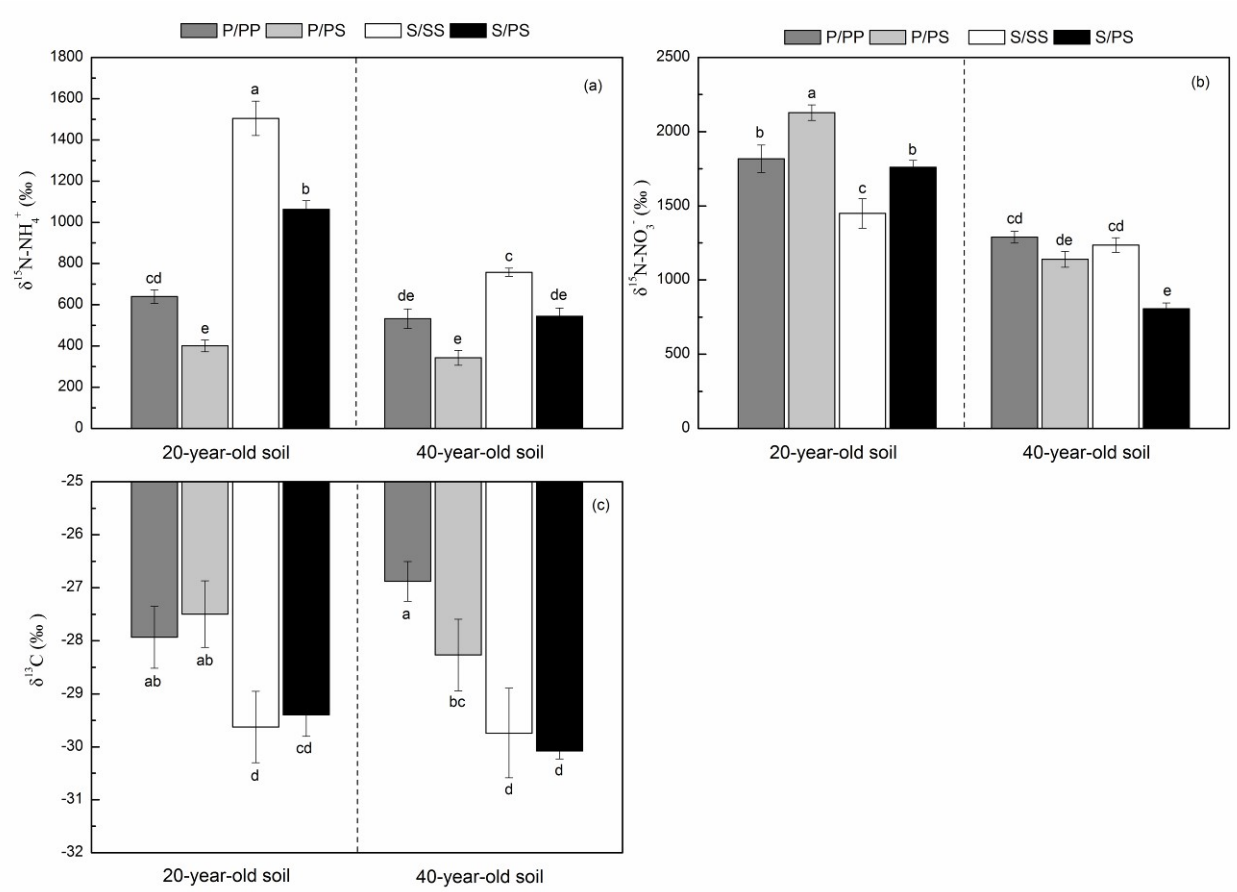

Leaf
Root
(a)
Starch content (mg g-1 DW)
(b)
Total sugar content (mg g-1 DW)
(c)
Sucrose content (mg g-1 DW)
(d)
Fructose content (mg g-1 DW)
P/PP
P/PS
S/SS
S/PS
20-year-old soil
40-year-old soil

Figure 5.


Leaf
Root
(a)
N content (mg g-1 DW)
(b)
P content (mg g-1 DW)
(c)
C : N ratio
(d)
N : P ratio
P/PP
P/PS
S/SS
S/PS
20-year-old soil
40-year-old soil

961 **Figure 6.**



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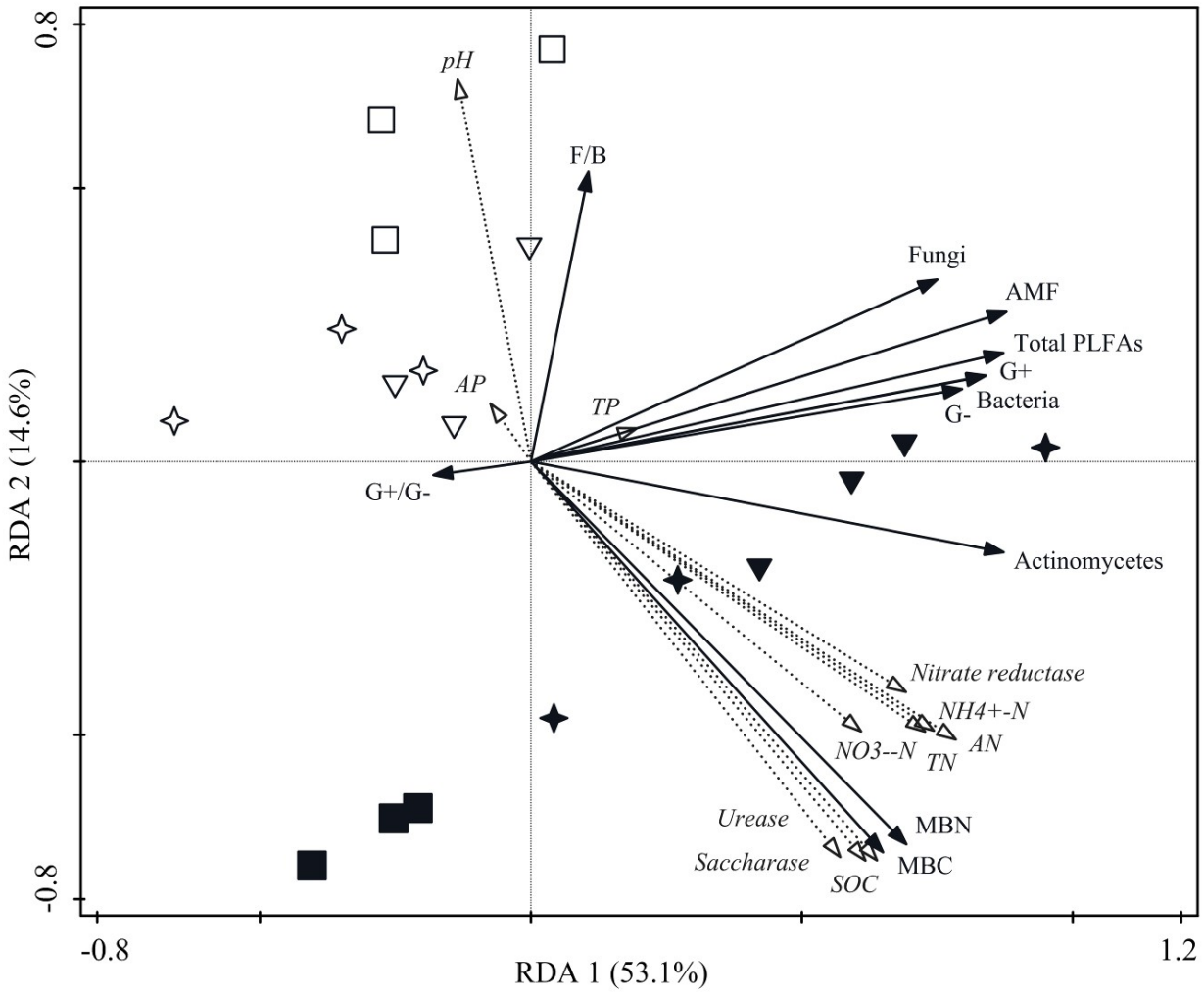
963

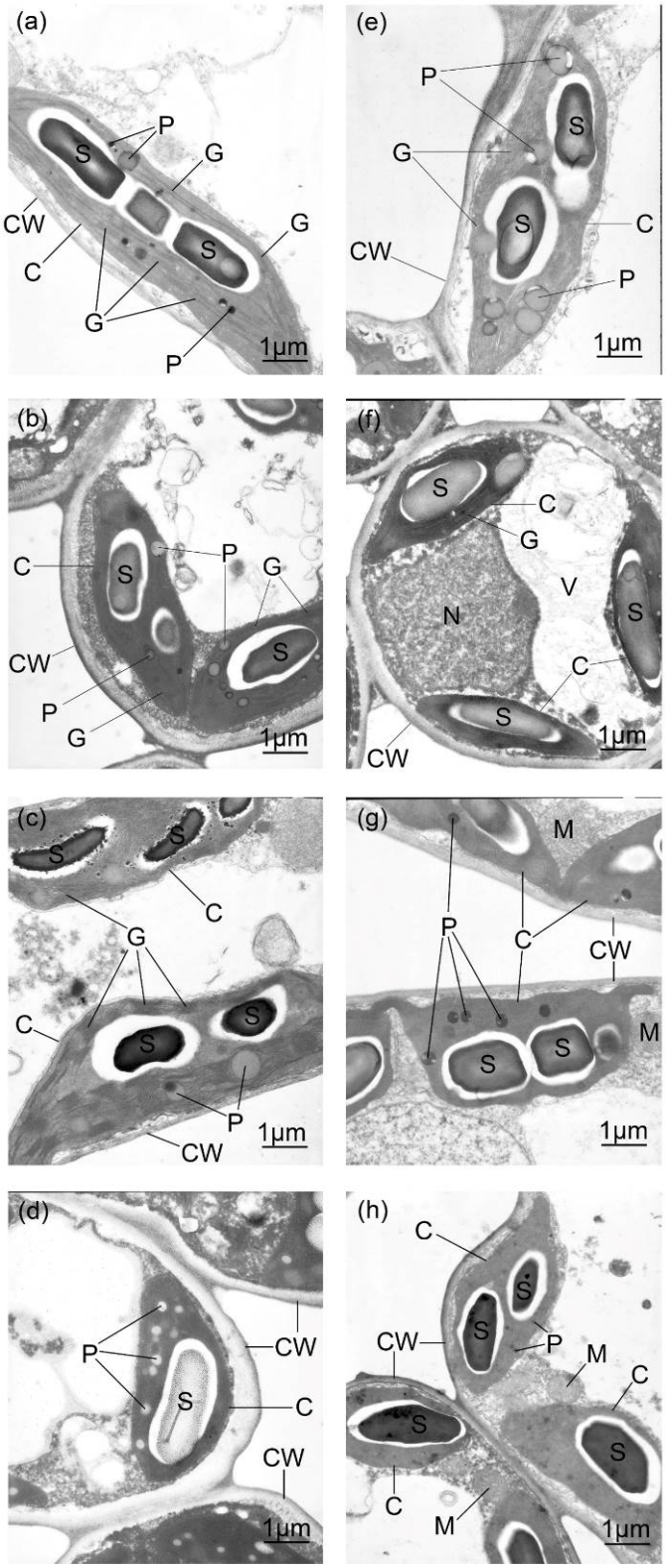
964 **Figure 7.**



965

966

Figure 8.


(a)
P
S
G
CW
G
C
S
G
P
1µm
(e)
P
S
G
S
C
CW
P
1µm
(b)
C
S
P
G
CW
S
P
G
1µm
(f)
S
C
G
V
N
S
C
S
CW
1µm
(c)
S
S
C
G
C
S
S
P
CW
1µm
(g)
M
P
C
CW
M
S
S
1µm
(d)
P
CW
S
C
CW
1µm
(h)
C
S
S
CW
P
M
C
S
S
C
M
1µm