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Biogeochemistry

11. May. 2021

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23 **Seasonality is more important than forest type in regulating the pool size and**

24 **composition of soil soluble N in temperate forests**

25 **Abstract**

26 Soil soluble nitrogen (N) is crucial to the N nutrition and productivity of plants. Consequently,

27 understanding the factors that affect its pool size and composition is of considerable

28 importance. Here, six typical forest types in northeast China were investigated to determine

29 the dynamics of soil soluble N across seasons and plant communities, and the potential

30 drivers. Soil free amino acids, NH_4^+, NO_3^-, dissolved organic N (DON) and a variety of soil

31 characteristics were measured over the growing season (from May to September). Seasonality

32 showed a stronger effect on the availability of soil inorganic N and free amino acids than

33 vegetation. The coefficients of variation of soil inorganic N, amino acid-N and the potential

34 drivers (moisture and DON) appeared to be greater for season, and the concentrations of

35 these available N sources tended to be higher at the beginning than at the height of growing

36 season. Potential soil drivers (*e.g.* moisture, microbial biomass-N and DON) and plant

37 phenology together drove the seasonal dynamics of inorganic N and amino acid-N. Arginine,

38 histidine, serine, leucine, aspartic acid, glycine, glutamic acid and proline composed the

39 dominant soil amino acid pool in the temperate forest soils. The basic amino acids (arginine

40 and histidine) were consistently dominant irrespective of vegetation and season, suggesting

41 that selective sorption by the soil solid phase could play an important role in regulating the

42 cycling of amino acid-N in these temperate forest ecosystems. This research indicates that

43   changes in local soil properties, and plant phenology caused by seasonality, exert a powerful

44   influence on the characteristics of plant-soil N cycling.

45   **Keywords**: Dominant amino acids; growing season; plant-soil N cycling; soil soluble N;

46   temperate forests

1 Introduction

48   Soil nitrogen (N) availability often limits the net primary productivity of most terrestrial

49   ecosystems (LeBauer and Treseder 2008). The availability of soil N is increasingly

50   influenced by N deposition (Bobbink et al. 2010; Liu et al. 2013), and understanding the

51   interaction of plant community and soil N cycling is very important. Soil soluble N in either

52   an organic form (*e.g.* amino acids, oligopeptides; Näsholm et al. 1998, 2009), or inorganic

53   form (*e.g.* NH_4^+-N and NO_3^--N) supplies N to plants and their associated symbionts and to

54   the wider soil microbial community. Although plants and microbes can access many forms of

55   N when grown in isolation, in natural systems they optimize their transport systems to reflect

56   the availability of different N sources. This can be viewed as an opportunistic response rather

57   than a preference for different N forms *per se* (Moreau et al. 2019). Further, the pool size and

58   composition of soil soluble N has been reported to regulate the relationship of inter-species

59   competition (Chapin et al. 1993; Harrison et al. 2007; Hill et al. 2011). Hence, the

60   investigation of the seasonal dynamics of soil soluble N across plant communities and the

61   potential drivers, is likely to prove important to understanding ecosystem function. Although

62   related research has been conducted in arctic (Weintraub and Schimel 2005), alpine (Lipson

63   et al. 1999b) and temperate grassland ecosystems (Warren and Taranto 2010), many gaps in

64   the knowledge on the temporal variability in soil N resources and the potential drivers still

65   remain, especially with respect to temperate forests. In the last two decades, soil amino

66   acid-N has been increasingly recognized as an important source of soluble N to plants

67   (Chapin et al. 1993; Hill et al. 2019b; Näsholm et al. 1998, 2009). The 20 common amino

68   acids that compose protein have different intrinsic properties, *e.g.* molecular weights,

69   structures and side-chain chemistry, which influence the behaviour of amino acid-N in soils

70   (Gonod et al. 2006; Rothstein 2010). Individual amino acids can therefore appear to be

71   dominant in the soil amino acid pool (Björk et al. 2007; Werdin-Pfisterer et al. 2012). Here

72   and throughout the paper the "dominant" soil amino acid means that it can be always detected

73   over time in the ecosystem.

74   The biochemical properties of soils are strongly influenced by plant communities (Björk et al.

75   2007; Wang et al. 2006; Werdin-Pfisterer et al. 2009), and it is logical to predict distinct N

76   cycling traits between plant communities. For example, some researchers have reported that

77   the concentration of total soil amino acids in the plant communities of late successional stage

78   forests are higher than that of early successional stages (Kielland et al. 2007;

79   Werdin-Pfisterer et al. 2009). Generally, plant communities can influence the availability of

80   soil soluble N, primarily through the following mechanisms: 1) soil temperature and moisture

81   could be changed by plant cover (D'Odorico et al. 2007; Roberts et al. 2009), potentially

82   influencing the activities of soil enzymes and microorganisms and the production of available

83   N. In particular, for soil moisture, a higher soil moisture content can increase the accessibility

84   of dissolved organic N (DON), which will be beneficial to the subsequent depolymerization

85   and mineralization of N; 2) the quantity and quality of litter input into soils could differ

86   among plant species. Conifer species are especially notable as they often create strongly

87   acidic soils, which do not favour nitrification (Killham 1990). Further, different foliage litters

88   contain different amounts and kinds of amino acids (Rajendran and Kathiresan 2000),

89   potentially leading to the production or leaching of some individual soil amino acids (Hicks

90   et al. 1991), while others may contain high levels of polyphenolics that repress mineralization

91   (Kraus et al. 2003); 3) the magnitude of N uptake could be species-specific (Kahmen et al.

92   2006; McKane et al. 2002). Different plant species or their symbionts may prefer to take up

93   specific N forms to satisfy their physiological needs (McKane et al. 2002); 4) roots can

94   excrete organic substances (Kuzyakov et al. 2007), including sugars (50–70% of total

95   exudate), carboxylic acids (20–30%) and amino acids (10–20%), which can stimulate

96   rhizosphere microbial activity and lead to soil organic matter decomposition, releasing

97   organic or inorganic N (Meier et al. 2017; Moreau et al. 2019).

98   As soil moisture, temperature and plant metabolism are determined by climate, however, the

99   four mechanisms described above are expected to be strongly regulated by season. Over the

100   growing season: first, air temperature and precipitation can change considerably. This is

101   especially true for the temperate forest ecosystems where soil temperature and moisture can

102   be significantly altered (Wang et al. 2006, 2013); second, the input of fresh litter to soils by

103   plants can change during the year, and N immobilization by soil microbes can be promoted

104   by the input of C-rich plant detritus. The soil microbial biomass can therefore also be

105   expected to fluctuate seasonally (Bardgett et al. 2007; Edwards et al. 2006); third, the

106   amounts and forms of available soil N and its uptake by plants can differ (McKane et al. 2002;

107   Stahl et al. 2011). The seasonal variation in the uptake of soil N by plants is likely to be very

108   strong, being high during the growing season and low when plants are not active (McKane et

109   al. 2002; Weintraub and Schimel 2005); last, the exudation rate of the organic substances by

110   plant roots can also be highly seasonally dynamic (Phillips et al. 2008). Therefore, the

111   production and consumption of soil inorganic N and amino acids are not only influenced by

112   plant community composition, but may also more deeply regulated by season. Under the

113   variation of seasonal climate between years, the temporal dynamics of these soil N sources

114   may even differ within a plant community (Warren and Taranto 2010).

115   The temperate forest ecosystems in northeast China are very functionally and spatially

116   important, accounting for 35% of the total forest area and 40% of the total forest biomass of

117   China (Wang et al. 2008). Over recent decades, anthropogenically-derived N deposition has

118   been shown to be substantial and has progressively risen in this region ($15-50$ kg N ha^{-1} yr^{-1};

119   Liu et al. 2013; Yu et al. 2019). This enhanced N deposition has changed the availability of

120   soil NH_4^+-N and NO_3^--N (Liu et al. 2013), which in turn may strongly influence species

121   composition or community succession (Bobbink et al. 2010; Hill et al. 2011; Stevens et al.

122   2010). In the context of this, exploring the cycling and feedback of plant-soil N in these

123 forests is important. On a monthly time scale during the growing season (from May to

124 September), the concentrations of soil free amino acid-N, NH_4^+-N, NO_3^--N, DON, microbial

125 biomass-N and a set of soil properties were quantified in typical forest types of northeast

126 China. Using this comprehensive data set, in these forest soils that are rich in organic matter

127 we aimed to explore: (1) the composition of the soil amino acid pool; (2) the dynamics of soil

128 inorganic N and amino acid-N across plant communities and seasons, and their potential

129 drivers. Specifically, we hypothesized that vegetation plays an important role in mediating

130 the pool size and the composition of soil inorganic N and amino acid-N, but that season could

131 be more influential than vegetation in influencing the availability of these soil available N

132 sources in the temperate forest ecosystems.

133 **2 Materials and methods**

134 **2.1 Study sites**

135 Two study sites, Liangshui National Natural Reserve (LNNR; 47°10′N, 128°53′E) and

136 Maoershan Forest Ecosystem Research Station (MFER; 45°24′N, 127°40′E), were used in the

137 study. LNNR is located in the Lesser Khingan Mountains, characterized by a temperate

138 monsoon climate with a mean annual temperature of -0.3 °C. The annual precipitation is 676

139 mm which mainly occurs in summer (Fig. 1). As one of the few virgin temperate forest

140 reserve areas in northeast China, LNNR has a variety of pristine forest types with a known

141 history of community succession and ecozones. Broad-leaved Korean pine (*Pinus koraiensis*)

142 mixed forest represents the typical zonal climax, while the forests of *Picea asperata* and

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143   *Abies nephrolepis* usually represent the topographic or edaphic climax at wet or swampy sites.

144   Since the clear-cutting of previous broad-leaved *Pinus koraiensis* mixed forest in the 1950s,

145   secondary birch (*Betula platyphylla*)-dominated stands which represent the naturally

146   regenerating forest type, have been established (see Qi et al. 2014 for more site details).

147   Based on this, four typical forest types were selected here for the study (Table 1).

148   MFER is located about 250 km south-west of LNNR, in the west branch of Zhangguangcai

149   Mountains which belongs to the Changbai Mountain System. Its climate is characterized by a

150   temperate continental monsoon, with a mean annual temperature of 2.8 °C and annual

151   precipitation of 629 mm, of which 80% falls in July and August (Fig. 1). Annual evaporation

152   determined by a Class A evaporation pan is 884 mm (Wang et al. 2006). The zonal climax

153   community here is also broad-leaved *Pinus koraiensis* mixed forest. However, due to strong

154   human interference in or before the 1930s the virgin forests have completely disappeared.

155   The current forest vegetation is dominated by broad-leaved secondary forests, with

156   regenerated *Pinus koraiensis* saplings under the canopy. Large areas of pure broad-leaved

157   and coniferous plantations have also been established. Two typical forest types were chosen

158   for the study at this site (Table 1).

159   **2.2 Soil sampling**

160   A 20 m × 30 m permanent plot was established in each forest type in 2016. Soil samples were

161   collected monthly from early May to late September, and sampling on rainy days or during

162   muddy periods was avoided. The actual sampling dates were, as follows: 6th May, 16th June,

163   15th July, 19th August and 21st September (for MFER site) or 23rd September (for LNNR site).

164   At each soil sampling time, 30 individual soil cores from the 0–10 cm mineral soil layer were

165   randomly taken within the plot of each forest type. The organic litter layer was removed by

166   hand before coring. The soil cores were cleared of plant debris and stones, placed into a

167   plastic bag, sealed and kept on ice during transport. Two teams simultaneously undertook the

168   field work, sampling on the early morning of each day. The sampling for any forest type was

169   completed within 2 h, followed by immediate transport to the laboratory. When soil samples

170   were brought back to the laboratory, six soil cores were randomly recombined into a

171   composite sample, making five composite samples for each forest type ($n=5$). These samples

172   were then stored at 4 °C for no more than 48 h.

173   **2.3 Laboratory procedures**

174   Soil samples were sieved to pass 2 mm. Soil $pH_{(water)}$ was measured on a 1:2 (weight/volume)

175   ratio, and moisture content was determined after drying at 105 °C for 24 h. Fresh soil

176   subsamples (4 g) were put into 50 mL centrifuge tubes, followed by the addition of 20 mL of

177   deionized water. The soil–water suspension was shaken at 220 rpm for 1 h, centrifuged at

178   16000 g for 15 min, and then vacuum-filtered through a 0.45 μm filter. The extracts were

179   stored at -20 °C until analysis. The concentrations of individual amino acids in solutions were

180   analyzed by reverse phase high performance liquid chromatography (HPLC) on an LC2000

181   (Techcomp, China) equipped with a Kromasil C_{18} HPLC column (150 mm × 4.6 mm, 5 μm).

182   6-aminoquinolyl-N-hyroxy-succinimidyl carbamate was applied to pre-derivatize the soil

183   extract solution (AQC; Liu 1994). The volume of sample injection was 10 μL, and the flow

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184   rate was 1.0 mL/min, with a column temperature of 37 °C and ultraviolet detection at 254

185   nm.

186   Soil dissolved N was measured using the potassium persulphate ($K_2S_2O_8$) oxidation method,

187   using distilled water as the extractant. DON was determined by the difference between

188   dissolved N and the resultant dissolved inorganic N (Bardgett et al. 2007). Soil microbial

189   biomass-N was determined by the chloroform fumigation-extraction method (Brookes et al.

190   1985). Soil NH_4^+ and NO_3^- extracted by 1 mol/L KCl were measured using an AA3 flow

191   injection auto-analyzer (Seal Analytical GmbH, Germany). The soil organic C and total N

192   contents were analyzed using a vario MACRO cube elemental analyzer (Elementar,

193   Germany).

194   **2.4 Statistical analyses**

195   Some amino acids were only detected in a minority of samples across the five months, so

196   only the "dominant" (see *Introduction*) soil amino acids were analyzed statistically as they

197   were deemed to be quantitatively important to the plant-soil system. Repeated measures

198   analysis of variance (ANOVA) was applied to examine the effects of forest type and month

199   on the concentration and the ratio of soil extracted N (SPSS 22.0; SPSS Inc., Chicago, IL,

200   USA). Principal component analysis (PCA) was performed to identify the variation in soil N

201   characteristics among forest types (OriginPro 2016; Originlab Corp., MA, USA). Multiple

202   regression analysis was used to determine the potential soil factors that drove the variation in

203   the availability of soil soluble N (SPSS 22.0; SPSS Inc., Chicago, IL, USA). The coefficients

204   of variation for soil soluble N, moisture and microbial biomass-N were calculated to compare

205　the degree of variation between forest type and season, and their differences were tested

206　through independent sample t-test (SPSS 22.0; SPSS Inc., Chicago, IL, USA).

207　**3 Results**

208　**3.1 Dynamics of soil soluble N and microbial biomass-N across forest types and months**

209　The concentrations of soil free amino acid-N, NH_4^+-N, NO_3^--N, DON, and microbial

210　biomass-N, showed marked variation among forest types and months (Fig. 2; Table S1).

211　There was a clear interaction between forest type and month (Table S1), with the

212　concentrations of soil soluble N or microbial biomass-N being highly variable from May to

213　September (Fig. 2). However, whether for soil soluble N or microbial biomass-N, the

214　concentrations of N tended to be the highest in May in comparison to the other months, with

215　only a few exceptions (Fig. 2). For secondary *Pinus koraiensis–Betula platyphylla* mixed

216　forest and *Fraxinus mandshurica* plantation, June exhibited the highest concentration of

217　NH_4^+-N while September exhibited the highest concentration of DON (Fig. 2b and d). For

218　*Picea asperata* plantation, July exhibited the highest microbial biomass-N (Fig. 2e).

219　Although the month that showed the lowest concentration of N varied between soil N

220　sources, these were concentrated between June and September (Fig. 2). Free amino acid-N

221　and NO_3^--N appeared to be the lowest in July than in other months, NH_4^+-N appeared to be

222　the lowest mostly in August, DON appeared to the lowest often in September but

223　occasionally in July, and microbial biomass-N showed the lowest concentrations in all

224　months except May (Fig. 2).

225　**3.2 Composition of dominant soil amino acids in temperate forests**

226   Forest type and month also appeared to influence the composition and concentration of the

227   dominant soil amino acids (Fig. 3; Table S1). Generally, eight dominant free amino acids

228   were detected in the temperate forest soils, and their concentrations followed the order:

229   arginine > histidine > serine > leucine > aspartic acid > glycine > glutamic acid > proline (Fig.

230   3). Except for arginine, histidine and aspartic acid, the other amino acids did not always

231   appear to be dominant across these forest soils (Fig. 3). This was especially obvious for

232   proline as it only appeared to be dominant in *Fraxinus mandshurica* plantation soils over the

233   growing season, with the concentration ranging from 0.10 to 0.26 μg N/g dry soil (Fig. 3h).

234   The concentrations of dominant soil amino acids also tended to be the highest in May than in

235   other months (Fig. 3). Nevertheless, for pristine *Abies nephrolepis* forest and *Picea asperata*

236   plantation, the concentrations of serine, aspartic acid and glutamic acid were the highest in

237   August (Fig. 3c, e and g). For secondary *Pinus koraiensis–Betula platyphylla* mixed forest,

238   the concentrations of glycine and glutamic acid were the highest in September (Fig. 3f and g).

239   The concentrations of dominant soil amino acids were the lowest mostly in July but

240   occasionally in June and August (Fig. 3). Pristine *Abies nephrolepis* forest was the only forest

241   type that showed the lowest concentrations of many dominant soil amino acids (arginine,

242   leucine, aspartic acid and glutamic acid) in June than in other months (Fig. 3a, d, e and g).

243   Meanwhile, in most cases the lowest concentrations were observed in August (Histidine,

244   aspartic acid, glutamic acid and proline) particularly in the secondary *Pinus*

245   *koraiensis–Betula platyphylla* mixed forest and *Fraxinus mandshurica* plantation (Fig. 3b, e,

246   g and h).

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3.3 Potential drivers of variation in soil inorganic N and free amino acids

Based on the stepwise analyses for the data of six forest types over five months, moisture, microbial biomass-N and DON were the most related soil factors that could explain the variation in the abundance of soil total free amino acids, NH_4^+, NO_3^-, arginine, histidine and aspartic acid (Table 2; Fig. 4). Other soil dominant amino acids were not analyzed as they did not occur in appreciable levels in all forest types. Soil total free amino acids, arginine and histidine were positively related to moisture which could explain 48.4%, 29.9% and 50.1% of the variation, respectively (Fig. 4a, d and e). Soil NH_4^+ was positively related to DON which could explain 25.6% of its variation (Fig. 4b). Soil NO_3^- and aspartic acid were both positively related to moisture and microbial biomass-N, which together could explain 31.6% and 46.0% of the variation, respectively (Fig. 4c and f).

3.4 Relationship among amino acid-N, inorganic N, DON and microbial biomass-N

Multivariate correlations among soil amino acid-N, inorganic N, DON and microbial biomass-N were analyzed by PCA (Fig. 5). The two main components together explained 70%, 79%, 77%, 78% and 77% of the variation in sample data for May, June, July, August and September, respectively (Fig. 5). The pattern of correlations of these soil biogenic N forms among the six forest types appeared to vary prominently over time. First, except for a few cases, different forest types showed a scattered pattern within a specific month (Fig. 5). For example, pristine broad-leaved *Pinus koraiensis* mixed forest and *Picea asperata* plantation clustered in September (Fig. 5e). These two forest types showed higher concentrations of soil histidine-N, serine-N, and lower NH_4^+-N, proline-N (not dominant),

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268   microbial biomass-N, and DON, which is opposite to *Fraxinus mandshurica* plantation (Fig.

269   5e); secondary *Pinus koraiensis–Betula platyphylla* mixed forest showed lower

270   concentrations of soil free amino acid-N, histidine-N and serine-N (not dominant) (Fig. 5e);

271   pristine *Abies nephrolepis* forest showed higher concentrations of soil glutamic acid-N,

272   glycine-N and leucine-N, whereas secondary *Picea asperata–Betula platyphylla* mixed forest

273   showed higher concentrations of soil NO_3^--N, arginine-N and aspartic acid-N (Fig. 5e).

274   Second, the distribution pattern of these forest types in the PCA appeared to differ over five

275   months. This could be well illustrated as the forest types that possessed similar soil N

276   characteristics (*i.e.* the clustered forest types) changed over time (Fig. 5).

277   **4 Discussion**

278   **4.1 Composition of dominant soil amino acids in temperate forest ecosystems**

279   An increasing body of research has employed amino acids as an organic N source to explore

280   ecologically crucial issues, *e.g.* organic N utilization (Näsholm et al. 1998), N niche

281   differentiation (McKane et al. 2002) and species coexistence (Ouyang et al. 2016). Here we

282   show that arginine, histidine, serine, leucine, aspartic acid, glycine, glutamic acid and proline

283   composed the dominant soil amino acids in the temperate forest ecosystems of northeast

284   China (Fig. 3). This provides a basis for further research that examines the importance of

285   dominant soil amino acid-N in plant nutrition within these forest ecosystems (Gao et al.

286   2019). The availability of soil amino acids is determined by the relative rates of production

287   and consumption; when the production rate exceeds the consumption rate, its availability

288   appears to increase and *vice versa*. The processes related to production and consumption

289   involve complex biochemical mechanisms. First, soil amino acids have various sources. The

290   breakdown of protein probably dominates (Jan et al. 2009), but dry and wet deposition

291   (Mopper and Zika 1987), vegetation stemflow and throughfall (Michalzik and Matzner

292   1999), root exudation and death (Jones and Darrah 1994), microbial turnover, and faunal

293   inputs (Hill et al. 2019a), also contribute. Second, amino acids have many sinks, including

294   the selective use by microorganisms and plants (Endres and Mercier 2003; Lipson et al.

295   1999a), mineralization (Jones and Kielland 2002) and leaching (Fischer et al. 2007). Probably

296   as a result of selective inputs or consumption, soil dominant amino acids appeared to be

297   distinct among forest types (Fig. 3) and also seemed different to the reports from other plant

298   ecosystems (Björk et al. 2007; Werdin-Pfisterer et al. 2012). Different forest types showed

299   different N cycling characteristics (Fig. 5), and the variation in the composition of soil amino

300   acids could partially reflect the variation in soil N cycling traits. This was especially

301   prominent for *Fraxinus mandshurica* plantation, as proline was dominant only in its soils (Fig.

302   3h). Although we cannot explain the phenomenon, it could illustrate that the composition of

303   soil dominant amino acids can indirectly reflect differences in soil-plant N cycling processes

304   between plant communities. Despite these differences in factors related to N cycling and the

305   variation in composition of the pool of amino acids, the basic amino acids, arginine and

306   histidine, appeared to continuously dominate in soils and on average accounted for 26% of

307   the free amino acids. This suggests that selective sorption of amino acids by the soil solid

308   phase could also play an important role in amino acid-N cycling in the temperate forest soils

309   (Rothstein 2010). The soils in the studied region mostly belong to dark brown forest soils,

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310   which are rich in organic matter (Table 1) and can have a high clay content (Zhang and Song

311   2005). This may partially contribute to the adsorption and prevalence of basic amino acids.

312   Additionally, aspartic acid belongs to the acidic amino acids which generally have a low

313   sorption potential on the soil solid phase relative to basic or neutral amino acids (Gonod et al.

314   2006), but it also dominated in the soil amino acid pool here (Fig. 3e). Some researchers

315   suggest that severed fine roots and mycorrhizal hyphae (Hobbie and Hobbie 2013) may lead

316   to the prevalence of aspartic acid (Senwo and Tabatabai 1998; Werdin-Pfisterer et al. 2009).

317   As described above, there were many potential factors that could affect the composition of

318   soil dominant amino acids. Based on the data collected here, we cannot explain explicitly

319   which factors induce the difference or similarity in the components of dominant soil amino

320   acids between forest types, but this deserves further research. Additionally, soil soluble N can

321   be primarily divided into three parts, which are mobile in soil solution, adsorbed to ion

322   exchange sites and spatially inaccessible due to isolation in microsites (Darrouzet-Nardi and

323   Weintraub 2014). In contrast to inorganic N (KCl extraction), deionized water as the

324   extractant for soil amino acids, which may underestimate the concentrations of some amino

325   acids that are prone to sorption on the soil's solid phase (*e.g.* histidine and arginine)

326   (Rothstein 2010; Werdin-Pfisterer et al. 2009). Nevertheless, free amino acids may be more

327   easily taken up by roots and microbes, representing more available N sources for organisms

328   than the adsorbed types.

329   **4.2 Seasonal dynamics of soil soluble N and potential drivers**

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330   Plant community type can significantly influence the turnover of soil C and N through the

331   change of soil biochemical conditions, *e.g.* pH (Killham 1990), the activity of enzymes and

332   the structure of the microbial community (Björk et al. 2007; Weand et al. 2010). The six

333   forest types chosen here were comprised of different plant species, and they were

334   characteristic of different growth stages, soil pH, microbial biomass, ratios of C to N and

335   relative moisture (Table 1; Fig. 2e). Some of the plant communities are in the early

336   successional stage (secondary *Pinus koraiensis–Betula platyphylla* mixed forest, secondary

337   *Picea asperata–Betula platyphylla* mixed forest and *Fraxinus mandshurica* plantation), while

338   the others are in a late successional stage (pristine broad-leaved *Pinus koraiensis* mixed forest,

339   *Picea asperata* plantation and pristine *Abies nephrolepis* forest). As hypothesized, the

340   concentration of soil soluble N was affected by plant communities (Figs. 2a, b, c, d and 3;

341   Table S1). This is in agreement with many studies from taiga forest (Kielland et al. 2007),

342   boreal forest (Werdin-Pfisterer et al. 2009) and tundra (Björk et al. 2007) ecosystems. As

343   there was interaction between forest type and month on the availability of soil soluble N

344   (Table S1), however, the distinction in the concentration of soil soluble N between plant

345   communities varied across months (Figs. 2 and 3). For instance, the concentration of total soil

346   amino acids in May was higher in pristine *Abies nephrolepis* forest than in secondary *Pinus*

347   *koraiensis–Betula platyphylla* mixed forest; in August, this was reversed (Fig. 2a). In contrast

348   to the total soil C or N pool in temperate forests, which is primarily comprised of organic

349   matter and can be stable for years to decades (Lützow et al. 2006), inorganic N and amino

350   acid-N can be strongly affected by many potential factors and can be easily consumed in soil

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351   solution (*e.g.* by roots, microbes or enzymes). Compared with season, the type of plant

352   community showed less influence on the availability of soil soluble N in the temperate forest

353   ecosystems of northeast China. This is different to the research from boreal and taiga forest

354   ecosystems where the concentrations of soil amino acids have been reported to increase over

355   the succession sequence of forest communities (Kielland et al. 2007; Werdin-Pfisterer et al.

356   2009). This difference suggests that the turnover characteristics of soil inorganic N or amino

357   acid-N could vary between forest ecosystems in different biomes.

358   In temperate forest ecosystems, belowground biological processes are extremely influenced

359   by the seasonal dynamics of soil temperature and moisture (Wang et al. 2006). From May to

360   September, the air temperature and precipitation changed considerably (Fig. 1), and the

361   characteristics of soil N cycling varied over the growing season (Fig. 5). The stronger

362   influence of season on the availability of soil inorganic N and free amino acids than forest

363   type, was primarily reflected in the following two aspects: 1) coefficients of variation of soil

364   inorganic N, free amino acids and the potential drivers (moisture and DON) tended to be

365   greater for month than for forest type, which was especially prominent for NO_3^- (Table 3). A

366   probable explanation could be because, compared with other forms of N, soil NO_3^- was more

367   prone to leaching induced by the seasonal change of precipitation; and 2) the concentrations

368   of soil inorganic N and free amino acids tended to be higher at the beginning than at the

369   height of growing season due to increased plant demand. From the onset to the height of

370   growing season, the concentration of soil free amino acid-N, NH_4^+-N and NO_3^--N decreased

371   by 62%, 57% and 72%, respectively (Fig. 6a, b and c). Both abiotic and biotic factors could

19

372 possibly lead to this phenomenon. The precipitation mostly occurred at the height of growing

373 season (Fig. 1), which could cause the leaching of soil soluble N especially for those that are

374 not readily adsorbed on the soil solid phase, *e.g.* acidic amino acids (Gonod et al. 2006) and

375 NO_3^-. However, as rainy days or muddy periods were avoided during soil sampling, so the

376 lower concentration of soil inorganic N, free amino acids and DON could not be directly

377 explained by the influence of precipitation. Based on stepwise analyses, the variations of soil

378 inorganic N and amino acids across forest types and months were potentially driven most by

379 soil moisture, followed by microbial biomass-N and DON (avg. 7.7% of which was free

380 amino acid-N), and the availability of inorganic N and amino acids were positively related to

381 these potential drivers (Table 2; Fig. 4). At the beginning of the growing season, the

382 concentration of soil moisture, microbial biomass-N and DON was 58%, 53% and 113%

383 higher than that at the height of growing season, respectively (Fig. 6d, e and f). In the early

384 May of northeast China, it was shortly after the periods of soil freezing and thawing (Wang et

385 al. 2013) which could release high amounts of water and N nutrients to the soil solution

386 (Grogan et al. 2014; Sanders-DeMott et al. 2018). The moist conditions could strengthen the

387 accessibility of DON sources to soil enzymes and microbes (Darrouzet-Nardi and Weintraub

388 2014), promoting the depolymerization and mineralization of N in soil solution. Therefore, as

389 reported by others (Bardgett et al. 2007; Hackl et al. 2004), we also suggest that soil moisture

390 is a very important factor for mediating the belowground processes related to soil N sources.

391 On the other hand, both the uptake of N by soil microbes and plant roots could also play an

392 important role in mediating the seasonal dynamics pattern of soil inorganic N and amino

393   acids. Nevertheless, the concentration of soil microbial biomass-N also decreased by 35%

394   from the onset to the height of growing season (Fig. 6f). As a storage pool of soil N, the

395   turnover of microbial N is frequently very rapid (three to five days; Kuzyakov and Xu 2013).

396   The available soil N will ultimately flow into the plants as they are more stable N storage

397   pool (Kuzyakov and Xu 2013). The phenology of plants is strongly influenced by seasonality,

398   driving the dynamic uptake of soil soluble N by roots (McKane et al. 2002; Stahl et al. 2011).

399   During the onset of the growing season, the N needed for plant growth can partially come

400   from the remobilization of plants' stored resources, and tree growth often starts before N

401   uptake by roots, reducing the N demand from the soils (Millard and Grelet 2010). Meanwhile,

402   the production of roots in the typical temperate forests of these regions can increase

403   significantly during the height of growing season (Quan et al. 2010). This can significantly

404   increase the uptake capacity and support the growth demand of plants for the limited N

405   resource. Thus, the reduction in the pools of available soil N during the height of growing

406   season in the temperate forest ecosystems most-likely results from the higher demand for N

407   nutrient by plants (Weintraub and Schimel 2005).

408   **5 Conclusions**

409   In the temperate forest ecosystems of northeast China, the pool size and composition of soil

410   soluble N were influenced more strongly by season than by vegetation. Soil potential drivers

411   (*e.g.* moisture, microbial biomass-N, DON) and the phenology of plants together drove the

412   seasonal dynamics of soil inorganic N and amino acid-N. Arginine, histidine, serine, leucine,

413   aspartic acid, glycine, glutamic acid and proline composed the dominant soil amino acids in

414　these temperate forest ecosystems; regardless of vegetation and season, however, basic amino

415　acids (arginine and histidine) dominated consistently, illustrating the potentially important

416　role of selective sorption by the soil solid phase in mediating soil amino acid-N cycling.

417　**Disclosure statement**: No potential conflict of interest was reported by the authors.

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639

Table 1 Selected characteristics of the study sites

Location	Type	Stand age (y)	Canopy closure	Elevation (m)	Slope position	Aspect	Slope (°)	Soil type (CST)	O hr. thk. (cm)	A hr. thk. (cm)	Organic C (g/kg)	Total N (g/kg)	C/N	pH	Relative moisture (%)
	Forest			Topography				Soil							
LNNR	PP	>250	0.80	436	Mid	SW	17	Bori-Udic Cambosols	5.0	18	96.2	5.97	16.1	5.79	58
	AN	>120	0.90	302	Valley	-	0	Molli-Orthic Gleyosols	6.5	12	71.4	4.41	17.76	5.66	90
	PB	55	0.75	374	Down	SW	10	Bori-Udic Cambosols	4.0	22	96.2	7.55	13.2	5.89	66
	PA	62	0.95	337	Down	W	3	Bori-Udic Cambosols	5.5	20	91.0	5.44	17.1	5.75	69
MFER	SP	55	0.95	385	Mid	SE	15	Bori-Udic Cambosols	3.5	15	86.2	5.32	17.4	6.10	52
	FP	50	0.85	332	Down	W	5	Bori-Udic Cambosols	2.5	20	105.1	9.81	11.0	6.19	56

650 **Table 2** Results of stepwise regression analysis.

Dependent variable	Variables entered	Sum of squares	df	F	P
FAA	Moisture content	202.0	1	28.3	< 0.001
NH_4^+	DON	297.1	1	10.9	0.003
NO_3^-	N_{mic} and moisture content	194.2	2	7.7	0.002
Arginine	Moisture content	2.9	1	13.7	0.001
Histidine	Moisture content	3.0	1	31.2	< 0.001
Aspartic acid	Moisture content and N_{mic}	1.4	2	13.7	< 0.001

651 Notes: Total free amino acids (FAA); dissolved organic N (DON); microbial biomass-N (N_{mic}).

652 Independent variables analyzed for stepwise regression analysis includes: organic C, N_{mic},

653 pH, total N, DON, moisture content and C/N ratio.

654 **Table 3** Coefficients of variation of soil soluble N, moisture

655 and microbial biomass-N across forest types and months.

| Soluble N | Coefficients of variation (%) | | | | |
	Forest type	Month	*df*	*t*	*P*
FAA	50 (26)	67 (22)	9	1.17	0.273
NH_4^+	55 (14)	72 (31)	9	1.17	0.271
NO_3^-	50 (8)	77 (20)	9	2.85	0.019
DON	39 (31)	61 (13)	9	1.59	0.145
Arginine	54 (24)	77 (30)	9	1.39	0.199
Histidine	58 (31)	72 (20)	9	0.87	0.407
Serine	62 (42)	94 (20)	7	1.37	0.214
Leucine	60 (23)	83 (12)	8	1.99	0.082
Aspartic acid	69 (35)	82 (30)	9	0.70	0.502
Glycine	52 (38)	83 (35)	6	1.16	0.292
Glutamic acid	57 (30)	79 (40)	7	0.95	0.375
Moisture	21 (9)	29 (8)	9	1.64	0.136
N_{mic}	44 (14)	38 (15)	9	− 0.73	0.483

656 Notes: Total free amino acids (FAA); dissolved organic N (DON); microbial biomass-N

657 (N_{mic}). Means (standard deviations) are shown.

658
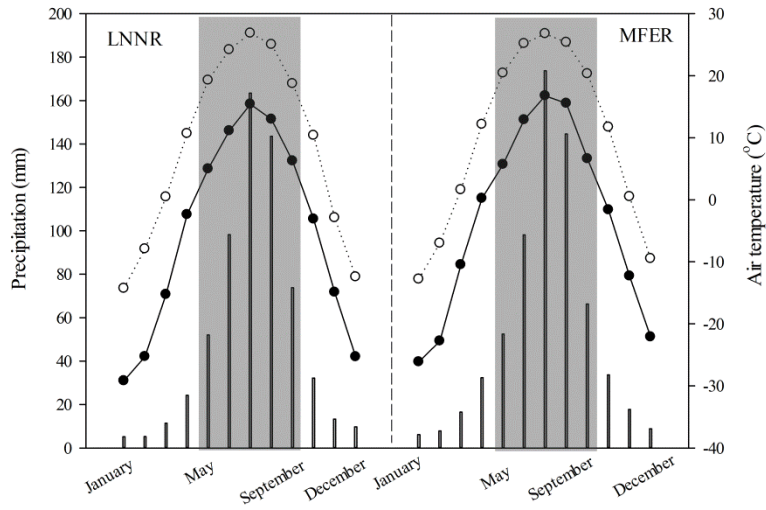
659　**Fig.1** Monthly averages of precipitation (bars), minimum (filled circles) and maximum (open circles)
660　air temperature in 2016 for Liangshui National Natural Reserve (LNNR) and Maoershan Forest
661　Ecosystem Research Station (MFER). The shaded area denotes the period of soil sampling. Data was
662　obtained from the local weather station for each site.
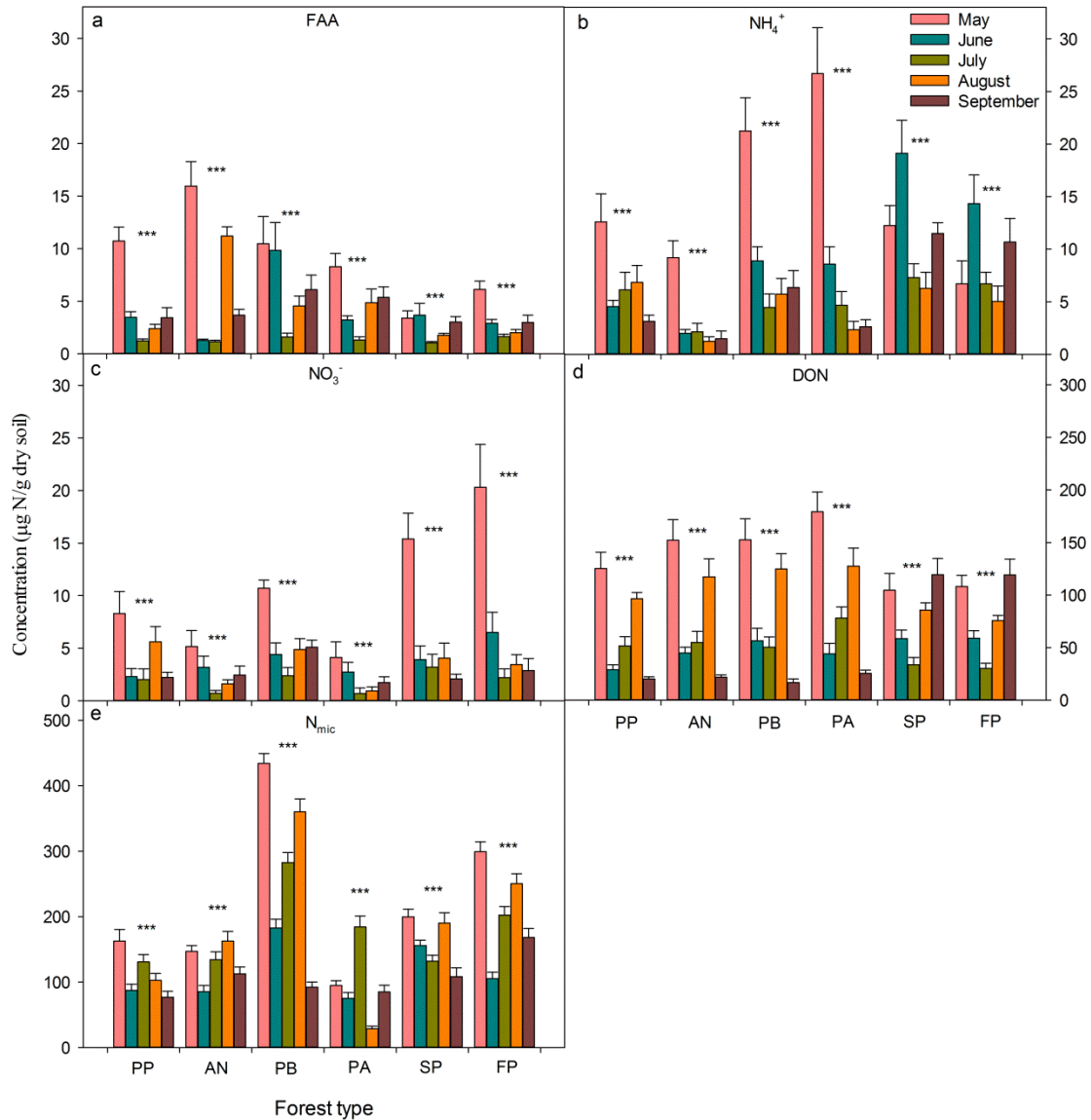
Fig. 2 Concentrations of soil total free amino acid-N (FAA; a), NH_4^+-N (b), NO_3^--N (c), dissolved organic N (DON; d) and microbial biomass-N (N_{mic}; e) over five months. Error bars show standard deviations (*n*=5). Pristine broad-leaved *Pinus koraiensis* mixed forest (PP); pristine *Abies nephrolepis* forest (AN); secondary *Picea asperata–Betula platyphylla* mixed forest (PB); *Picea asperata* plantation (PA); secondary *Pinus koraiensis–Betula platyphylla* mixed forest (SP); *Fraxinus mandshurica* plantation (FP). *** indicates significant difference between months within a forest at the *p* < 0.001 level.
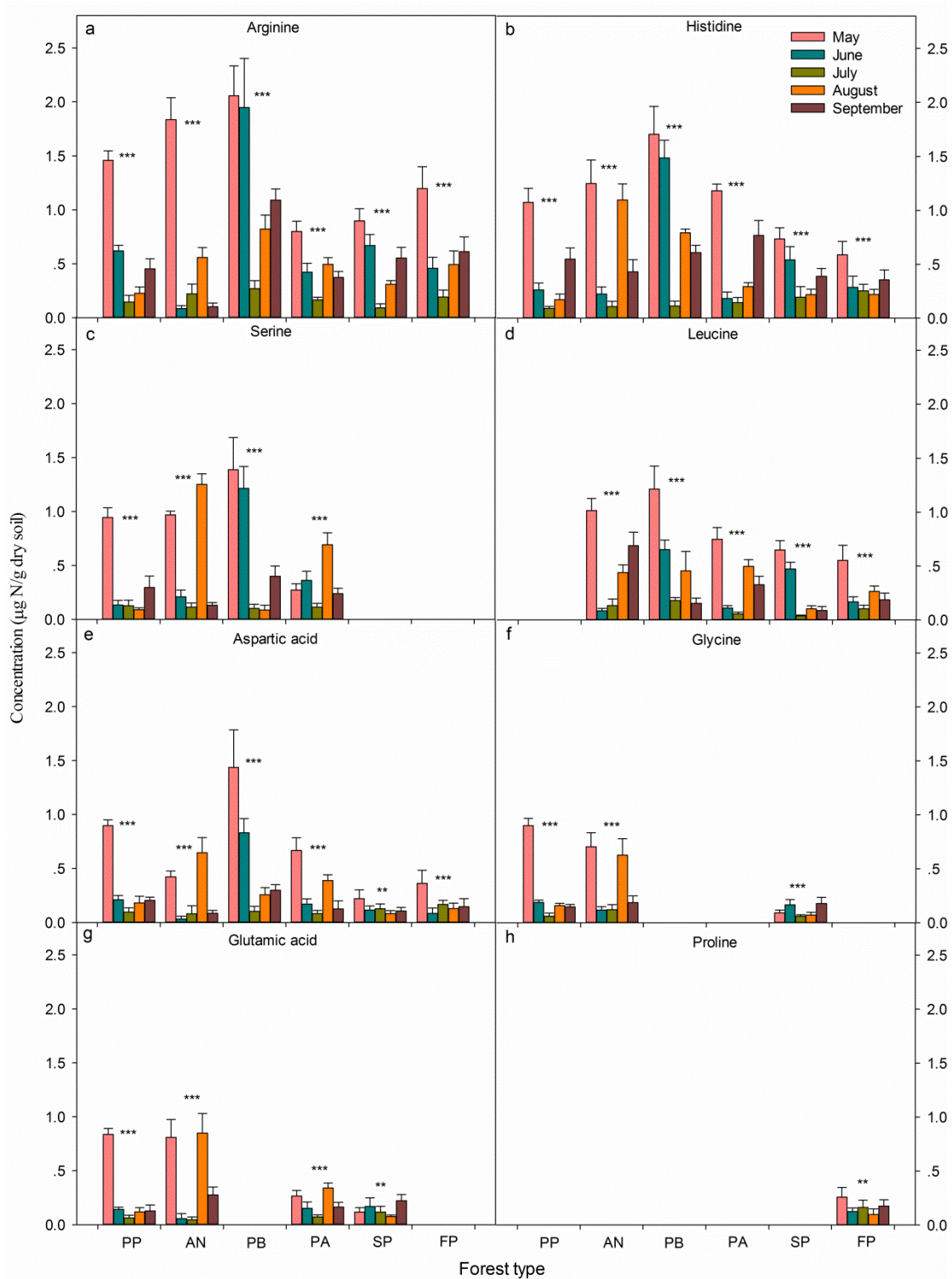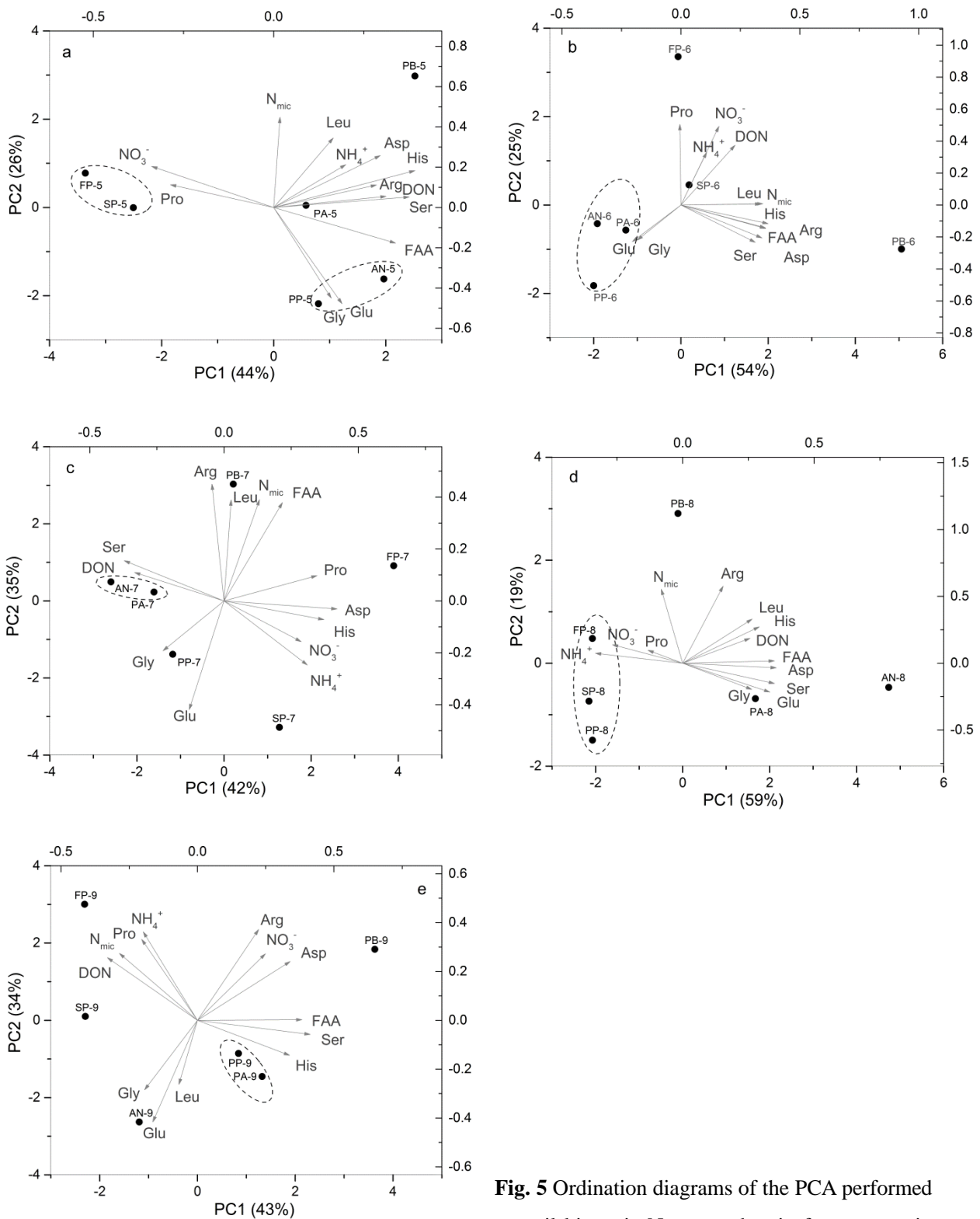
Fig. 3 Concentrations of dominant soil free amino acids in pristine broad-leaved *Pinus koraiensis* mixed forest (PP), pristine *Abies nephrolepis* forest (AN), secondary *Picea asperata–Betula platyphylla* mixed forest (PB), *Picea asperata* plantation (PA), secondary *Pinus koraiensis–Betula platyphylla* mixed forest (SP), and *Fraxinus mandshurica* plantation (FP) over the growing season. May (5); June (6); July (7); August (8); September (9). Error bars show standard deviations (*n*=5). ** and *** indicates significant difference between months within a forest at the *p* < 0.01 and 0.001

676   level, respectively.
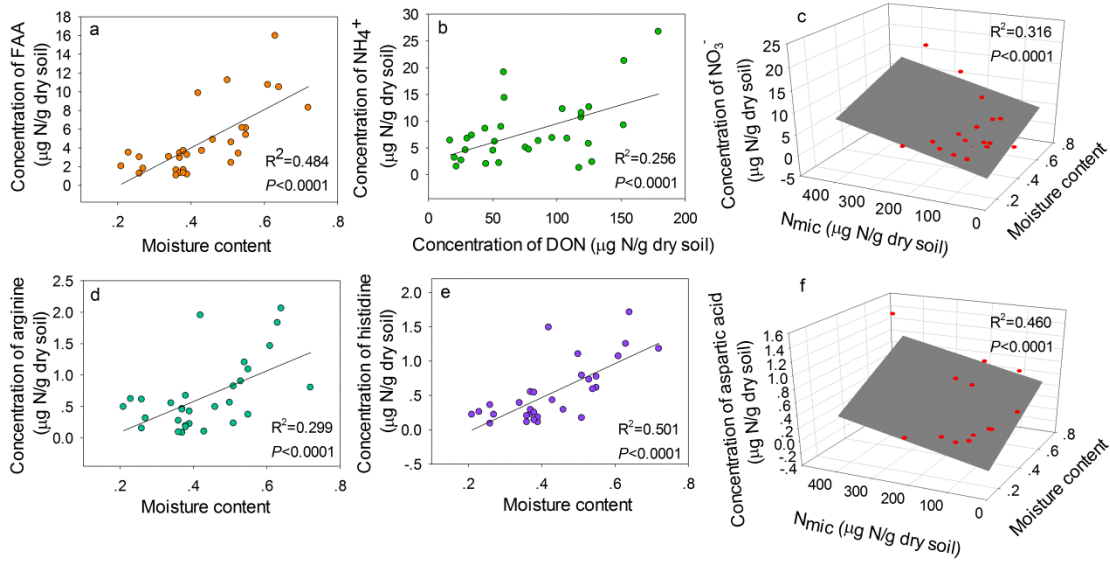
Fig. 4 Relationships between soil soluble N (total free amino acids, FAA, a; NH_4^+, b; NO_3^-, c; arginine, d; histidine, e; aspartic acid, f) and the related factors obtained from stepwise regression analyses. Microbial biomass-N, N_{mic}. Each colored circle indicates the monthly mean of a forest type (n=30).

Fig. 5 Ordination diagrams of the PCA performed on soil biogenic N across the six forest types in May (a), June (b), July (c), August (d), and September (e). Pristine broad-leaved *Pinus koraiensis* mixed forest (PP); pristine *Abies nephrolepis* forest (AN); secondary *Picea asperata–Betula platyphylla* mixed forest (PB); *Picea asperata* plantation (PA); secondary *Pinus koraiensis–Betula platyphylla* mixed forest (SP); *Fraxinus mandshurica* plantation (FP). Arginine (Arg); Histidine

687   (His); Serine (Ser); Leucine (Leu); Aspartic acid (Asp); Glycine (Gly); Glutamic acid (Glu); Proline

688   (Pro); total free amino acids (FAA); dissolved organic N (DON); microbial biomass-N (N_{mic}).

689   Numbers linked with forest types indicate corresponding months. Forest types showing similar soil

690   N characteristics are indicated by the ovals.
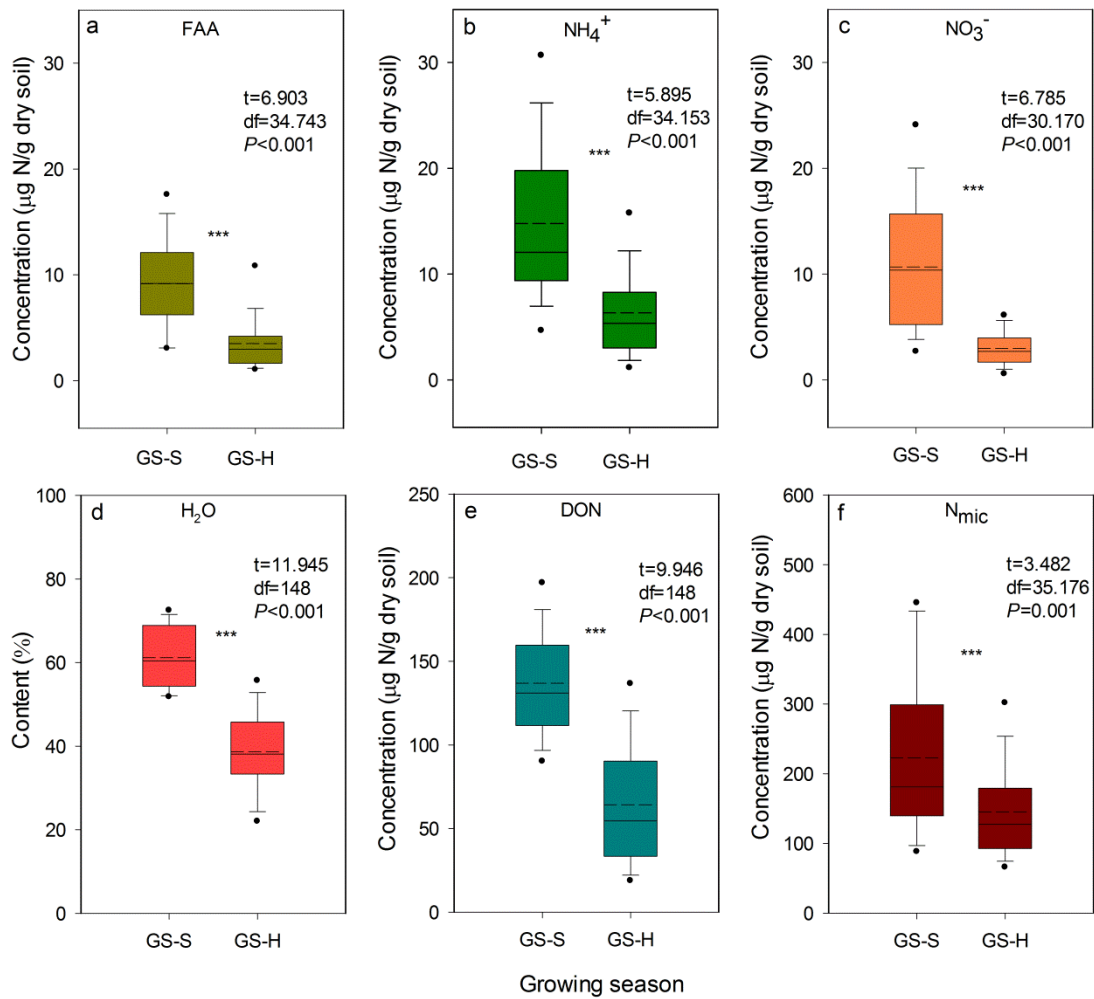
Fig. 6 Concentrations of soil total free amino acid-N (FAA; a), NH_4^+-N (b), NO_3^--N (c), moisture (H_2O; d), dissolved organic N (DON; e) and microbial biomass-N (N_{mic}; f) in temperate forests during the start of growing season (GS-S; May; $n=30$) and the height of growing season (GS-H; June, July, August and September; $n=120$). The boundaries of the box indicate the 25% and 75% percentiles, the whiskers indicate the 10% and 90% percentiles, and the points indicate the 5% and 95% percentiles, respectively. The solid line in the box marks the median, and the dotted line marks the mean. *** indicates significant difference at the $p < 0.001$ level.

Table S1 Repeated measures analysis of variance for the effects of forest type

and month on the concentration of soil extracted N.

N source	Forest type			Month			Forest type × Month		
	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
FAA	5	46.1	< 0.001	1.8	230.0	< 0.001	8.8	33.1	< 0.001
NH_4^+	5	16.6	< 0.001	2.3	444.0	< 0.001	11.4	88.5	< 0.001
NO_3^-	5	15.6	< 0.001	1.8	475.7	< 0.001	8.8	44.9	< 0.001
DON	5	3.8	< 0.011	2.3	944.5	< 0.001	11.4	97.7	< 0.001
Arginine	5	147.3	< 0.001	2.4	303.5	< 0.001	11.9	25.9	< 0.001
Histidine	5	111.8	< 0.001	2.4	298.1	< 0.001	12.0	37.9	< 0.001
Serine	3	51.8	< 0.001	2.5	170.0	< 0.001	7.6	80.9	< 0.001
Leucine	4	39.6	< 0.001	2.7	261.2	< 0.001	10.6	26.1	< 0.001
Aspartic acid	5	144.9	< 0.001	1.8	156.5	< 0.001	8.9	28.3	< 0.001
Glycine	2	64.4	< 0.001	4	152.1	< 0.001	8	68.3	< 0.001
Glutamic acid	3	61.5	< 0.001	2.5	121.3	< 0.001	7.5	50.1	< 0.001
Proline	-	-	-	4	4.0	0.020	-	-	-
N_{mic}	5	974.7	< 0.001	4	433.2	< 0.001	20	100.7	< 0.001

Note: Total free amino acids (FAA); dissolved organic N (DON); microbial biomass-N (N_{mic}).