



# Seasonality is more important than forest type in regulating the pool size and composition of soil soluble N in temperate forests

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**Abstract** Soil soluble nitrogen (N) is crucial to the N nutrition and productivity of plants. Consequently, understanding the factors that affect its pool size and composition is of considerable importance. Here, six typical forest types in northeast China were investigated to determine the dynamics of soil soluble N across seasons and plant communities, and the potential drivers. Soil free amino acids,  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , dissolved organic N (DON) and a variety of soil characteristics were measured over the growing

season (from May to September). Seasonality showed a stronger effect on the availability of soil inorganic N and free amino acids than vegetation. The coefficients of variation of soil inorganic N, amino acid-N and the potential drivers (moisture and DON) appeared to be greater for season, and the concentrations of these available N sources tended to be higher at the beginning than at the height of growing season. Potential soil drivers (*e.g.* moisture, microbial biomass-N and DON) and plant phenology together drove the seasonal dynamics of inorganic N and amino acid-N. Arginine, histidine, serine, leucine, aspartic acid, glycine, glutamic acid and proline composed the dominant soil amino acid pool in the temperate forest soils. The basic amino acids (arginine and histidine) were consistently dominant irrespective of vegetation

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and season, suggesting that selective sorption by the soil solid phase could play an important role in regulating the cycling of amino acid-N in these temperate forest ecosystems. This research indicates that changes in local soil properties, and plant phenology caused by seasonality, exert a powerful influence on the characteristics of plant-soil N cycling.

**Keywords** Dominant amino acids · Growing season · Plant-soil N cycling · Soil soluble N · Temperate forests

## Introduction

Soil nitrogen (N) availability often limits the net primary productivity of most terrestrial ecosystems (LeBauer and Treseder 2008). The availability of soil N is increasingly influenced by N deposition (Bobbink et al. 2010; Liu et al. 2013), and understanding the interaction of plant community and soil N cycling is very important. Soil soluble N in either an organic form (*e.g.* amino acids, oligopeptides; Näsholm et al. 1998, 2009), or inorganic form (*e.g.*  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N) supplies N to plants and their associated symbionts and to the wider soil microbial community. Although plants and microbes can access many forms of N when grown in isolation, in natural systems they optimize their transport systems to reflect the availability of different N sources. This can be viewed as an opportunistic response rather than a preference for different N forms per se (Moreau et al. 2019). Further, the pool size and composition of soil soluble N has been reported to regulate the relationship of interspecies competition (Chapin et al. 1993; Harrison et al. 2007; Hill et al. 2011). Hence, the investigation of the seasonal dynamics of soil soluble N across plant communities and the potential drivers, is likely to prove important to understanding ecosystem function. Although related research has been conducted in arctic (Weintraub and Schimel 2005), alpine (Lipson et al. 1999b) and temperate grassland ecosystems (Warren and Taranto 2010), many gaps in the knowledge on the temporal variability in soil N resources and the potential drivers still remain, especially with respect to temperate forests. In the last two decades, soil amino acid-N has been increasingly recognized as an important source of soluble N to plants (Chapin et al.

1993; Hill et al. 2019b; Näsholm et al. 1998, 2009). The 20 common amino acids that compose protein have different intrinsic properties, *e.g.* molecular weights, structures and side-chain chemistry, which influence the behaviour of amino acid-N in soils (Gonod et al. 2006; Rothstein 2010). Individual amino acids can therefore appear to be dominant in the soil amino acid pool (Björk et al. 2007; Werdin-Pfisterer et al. 2012). Here and throughout the paper the “dominant” soil amino acid means that it can be always detected over time in the ecosystem.

The biochemical properties of soils are strongly influenced by plant communities (Björk et al. 2007; Wang et al. 2006; Werdin-Pfisterer et al. 2009), and it is logical to predict distinct N cycling traits between plant communities. For example, some researchers have reported that the concentration of total soil amino acids in the plant communities of late successional stage forests are higher than that of early successional stages (Kielland et al. 2007; Werdin-Pfisterer et al. 2009). Generally, plant communities can influence the availability of soil soluble N, primarily through the following mechanisms: (1) soil temperature and moisture could be changed by plant cover (D’Odorico et al. 2007; Roberts et al. 2009), potentially influencing the activities of soil enzymes and microorganisms and the production of available N. In particular, for soil moisture, a higher soil moisture content can increase the accessibility of dissolved organic N (DON), which will be beneficial to the subsequent depolymerization and mineralization of N; (2) the quantity and quality of litter input into soils could differ among plant species. Conifer species are especially notable as they often create strongly acidic soils, which do not favour nitrification (Killham 1990). Further, different foliage litters contain different amounts and kinds of amino acids (Rajendran and Kathiresan 2000), potentially leading to the production or leaching of some individual soil amino acids (Hicks et al. 1991), while others may contain high levels of polyphenolics that repress mineralization (Kraus et al. 2003); (3) the magnitude of N uptake could be species-specific (Kahmen et al. 2006; McKane et al. 2002). Different plant species or their symbionts may prefer to take up specific N forms to satisfy their physiological needs (McKane et al. 2002); (4) roots can excrete organic substances (Kuzyakov et al. 2007), including sugars (50–70% of total exudate), carboxylic acids (20–30%) and amino acids

(10–20%), which can stimulate rhizosphere microbial activity and lead to soil organic matter decomposition, releasing organic or inorganic N (Meier et al. 2017; Moreau et al. 2019).

As soil moisture, temperature and plant metabolism are determined by climate, however, the four mechanisms described above are expected to be strongly regulated by season. Over the growing season: first, air temperature and precipitation can change considerably. This is especially true for the temperate forest ecosystems where soil temperature and moisture can be significantly altered (Wang et al. 2006, 2013); second, the input of fresh litter to soils by plants can change during the year, and N immobilization by soil microbes can be promoted by the input of C-rich plant detritus. The soil microbial biomass can therefore also be expected to fluctuate seasonally (Bardgett et al. 2007; Edwards et al. 2006); third, the amounts and forms of available soil N and its uptake by plants can differ (McKane et al. 2002; Stahl et al. 2011). The seasonal variation in the uptake of soil N by plants is likely to be very strong, being high during the growing season and low when plants are not active (McKane et al. 2002; Weintraub and Schimel 2005); last, the exudation rate of the organic substances by plant roots can also be highly seasonally dynamic (Phillips et al. 2008). Therefore, the production and consumption of soil inorganic N and amino acids are not only influenced by plant community composition, but may also more deeply regulated by season. Under the variation of seasonal climate between years, the temporal dynamics of these soil N sources may even differ within a plant community (Warren and Taranto 2010).

The temperate forest ecosystems in northeast China are very functionally and spatially important, accounting for 35% of the total forest area and 40% of the total forest biomass of China (Wang et al. 2008). Over recent decades, anthropogenically-derived N deposition has been shown to be substantial and has progressively risen in this region (15–50 kg N/ha/year; Liu et al. 2013; Yu et al. 2019). This enhanced N deposition has changed the availability of soil  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N (Liu et al. 2013), which in turn may strongly influence species composition or community succession (Bobbink et al. 2010; Hill et al. 2011; Stevens et al. 2010). In the context of this, exploring the cycling and feedback of plant-soil N in these forests is important. On a monthly time scale during

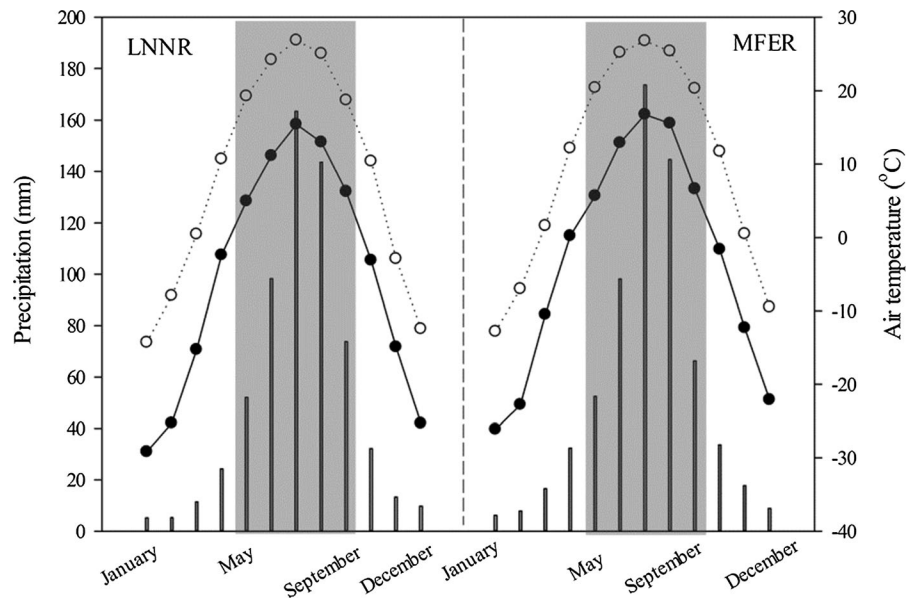
the growing season (from May to September), the concentrations of soil free amino acid-N,  $\text{NH}_4^+$ -N,  $\text{NO}_3^-$ -N, DON, microbial biomass-N and a set of soil properties were quantified in typical forest types of northeast China. Using this comprehensive data set, in these forest soils that are rich in organic matter we aimed to explore: (1) the composition of the soil amino acid pool; (2) the dynamics of soil inorganic N and amino acid-N across plant communities and seasons, and their potential drivers. Specifically, we hypothesized that vegetation plays an important role in mediating the pool size and the composition of soil inorganic N and amino acid-N, but that season could be more influential than vegetation in influencing the availability of these soil available N sources in the temperate forest ecosystems.

## Materials and methods

### Study sites

Two study sites, Liangshui National Natural Reserve (LNNR; 47°10'N, 128°53'E) and Maoershan Forest Ecosystem Research Station (MFER; 45°24'N, 127°40'E), were used in the study. LNNR is located in the Lesser Khingan Mountains, characterized by a temperate monsoon climate with a mean annual temperature of  $-0.3\text{ }^\circ\text{C}$ . The annual precipitation is 676 mm which mainly occurs in summer (Fig. 1). As one of the few virgin temperate forest reserve areas in northeast China, LNNR has a variety of pristine forest types with a known history of community succession and ecozones. Broad-leaved Korean pine (*Pinus koraiensis*) mixed forest represents the typical zonal climax, while the forests of *Picea asperata* and *Abies nephrolepis* usually represent the topographic or edaphic climax at wet or swampy sites. Since the clear-cutting of previous broad-leaved *P. koraiensis* mixed forest in the 1950s, secondary birch (*Betula platyphylla*)-dominated stands which represent the naturally regenerating forest type, have been established (see Qi et al. 2014 for more site details). Based on this, four typical forest types were selected here for the study (Table 1).

MFER is located about 250 km south-west of LNNR, in the west branch of Zhangguangcai Mountains which belongs to the Changbai Mountain System. Its climate is characterized by a temperate



**Fig. 1** Monthly averages of precipitation (bars), minimum (filled circles) and maximum (open circles) air temperature in 2016 for Liangshui National Natural Reserve (LNNR) and

Maershan Forest Ecosystem Research Station (MFER). The shaded area denotes the period of soil sampling. Data was obtained from the local weather station for each site

continental monsoon, with a mean annual temperature of 2.8 °C and annual precipitation of 629 mm, of which 80% falls in July and August (Fig. 1). Annual evaporation determined by a Class A evaporation pan is 884 mm (Wang et al. 2006). The zonal climax community here is also broad-leaved *P. koraiensis* mixed forest. However, due to strong human interference in or before the 1930s the virgin forests have completely disappeared. The current forest vegetation is dominated by broad-leaved secondary forests, with regenerated *P. koraiensis* saplings under the canopy. Large areas of pure broad-leaved and coniferous plantations have also been established. Two typical forest types were chosen for the study at this site (Table 1).

### Soil sampling

A 20 m × 30 m permanent plot was established in each forest type in 2016. Soil samples were collected monthly from early May to late September, and sampling on rainy days or during muddy periods was avoided. The actual sampling dates were, as follows: 6th May, 16th June, 15th July, 19th August and 21st September (for MFER site) or 23rd September (for LNNR site). At each soil sampling time, 30 individual

soil cores from the 0 to 10 cm mineral soil layer were randomly taken within the plot of each forest type. The organic litter layer was removed by hand before coring. The soil cores were cleared of plant debris and stones, placed into a plastic bag, sealed and kept on ice during transport. Two teams simultaneously undertook the field work, sampling on the early morning of each day. The sampling for any forest type was completed within 2 h, followed by immediate transport to the laboratory. When soil samples were brought back to the laboratory, six soil cores were randomly recombined into a composite sample, making five composite samples for each forest type ( $n = 5$ ). These samples were then stored at 4 °C for no more than 48 h.

### Laboratory procedures

Soil samples were sieved to pass 2 mm. Soil  $\text{pH}_{(\text{water})}$  was measured on a 1:2 (weight/volume) ratio, and moisture content was determined after drying at 105 °C for 24 h. Fresh soil subsamples (4 g) were put into 50 mL centrifuge tubes, followed by the addition of 20 mL of deionized water. The soil–water suspension was shaken at 220 rpm for 1 h, centrifuged at  $16,000 \times g$  for 15 min, and then vacuum-filtered

**Table 1** Selected characteristics of the study sites

Location	Forest			Topography			
	Type	Stand age (y)	Canopy closure	Elevation (m)	Slope position	Aspect	Slope (°)
LNNR	PP	> 250	0.80	436	Mid	SW	17
	AN	> 120	0.90	302	Valley	–	0
	PB	55	0.75	374	Down	SW	10
	PA	62	0.95	337	Down	W	3
MFER	SP	55	0.95	385	Mid	SE	15
	FP	50	0.85	332	Down	W	5

Location	Soil							
	Soil type (CST)	O hr thk (cm)	A hr thk (cm)	Organic C (g/kg)	Total N (g/kg)	C/N	pH	Relative moisture (%)
LNNR	Bori-Udic Cambosols	5.0	18	96.2	5.97	16.1	5.79	58
	Molli-Orthic Gleysols	6.5	12	71.4	4.41	17.6	5.66	90
	Bori-Udic Cambosols	4.0	22	96.2	7.55	13.2	5.89	66
	Bori-Udic Cambosols	5.5	20	91.0	5.44	17.1	5.75	69
MFER	Bori-Udic Cambosols	3.5	15	86.2	5.32	17.4	6.10	52
	Bori-Udic Cambosols	2.5	20	105.1	9.81	11.0	6.19	56

Relative moisture represents the ratio of field soil moisture content (w %) to saturated moisture content (w %). Soil organic C, total N, C/N, pH, and relative humidity were determined with 0–10 cm soil, and are means across 5 months ( $n = 25$ )

LNNR Liangshui National Natural Reserve; MFER Maoershan Forest Ecosystem Research Station; PP pristine broad-leaved *Pinus koraiensis* mixed forest, composed of 70% pine + 30% broad-leaved; AN pristine *Abies nephrolepis* forest, composed of 90% fir + 10% birch; PB secondary *Picea asperata*–*Betula platyphylla* mixed forest, composed of 85% birch + 15% spruce; PA *Picea asperata* plantation, with less than 10% birch invaded; SP secondary *Pinus koraiensis*–*Betula platyphylla* mixed forest, the broad-leaved secondary forest with regenerated *Pinus koraiensis* saplings under canopy; FP *Fraxinus mandshurica* plantation; SW southwest; W west; CST Chinese Soil Taxonomy; O hr.thk. O horizon thickness; A hr.thk. A horizon thickness

through a 0.45  $\mu\text{m}$  filter. The extracts were stored at  $-20\text{ }^{\circ}\text{C}$  until analysis. The concentrations of individual amino acids in solutions were analyzed by reverse phase high performance liquid chromatography (HPLC) on an LC2000 (Techcomp, China) equipped with a Kromasil C<sub>18</sub> HPLC column (150 mm  $\times$  4.6 mm, 5  $\mu\text{m}$ ). 6-aminoquinolyl-N-hydroxy-succinimidyl carbamate was applied to pre-derivatize the soil extract solution (AQC; Liu 1994). The volume of sample injection was 10  $\mu\text{L}$ , and the flow rate was 1.0 mL/min, with a column temperature of 37  $^{\circ}\text{C}$  and ultraviolet detection at 254 nm.

Soil dissolved N was measured using the potassium persulphate ( $\text{K}_2\text{S}_2\text{O}_8$ ) oxidation method, using distilled water as the extractant. DON was determined by the difference between dissolved N and the resultant dissolved inorganic N (Bardgett et al. 2007). Soil microbial biomass-N was determined by the chloroform fumigation-extraction method (Brookes et al.

1985). Soil  $\text{NH}_4^+$  and  $\text{NO}_3^-$  extracted by 1 mol/L KCl were measured using an AA3 flow injection auto-analyzer (Seal Analytical GmbH, Germany). The soil organic C and total N contents were analyzed using a vario MACRO cube elemental analyzer (Elementar, Germany).

#### Statistical analyses

Some amino acids were only detected in a minority of samples across the 5 months, so only the “dominant” (see Introduction) soil amino acids were analyzed statistically as they were deemed to be quantitatively important to the plant-soil system. Repeated measures analysis of variance (ANOVA) was applied to examine the effects of forest type and month on the concentration and the ratio of soil extracted N (SPSS 22.0; SPSS Inc., Chicago, IL, USA). Principal component analysis (PCA) was performed to identify the

variation in soil N characteristics among forest types (OriginPro 2016; Originlab Corp., MA, USA). Multiple regression analysis was used to determine the potential soil factors that drove the variation in the availability of soil soluble N (SPSS 22.0; SPSS Inc., Chicago, IL, USA). The coefficients of variation for soil soluble N, moisture and microbial biomass-N were calculated to compare the degree of variation between forest type and season, and their differences were tested through independent sample t-test (SPSS 22.0; SPSS Inc., Chicago, IL, USA).

## Results

### Dynamics of soil soluble N and microbial biomass-N across forest types and months

The concentrations of soil free amino acid-N,  $\text{NH}_4^+$ -N,  $\text{NO}_3^-$ -N, DON, and microbial biomass-N, showed marked variation among forest types and months (Fig. 2; Table S1). There was a clear interaction between forest type and month (Table S1), with the concentrations of soil soluble N or microbial biomass-N being highly variable from May to September (Fig. 2). However, whether for soil soluble N or microbial biomass-N, the concentrations of N tended to be the highest in May in comparison to the other months, with only a few exceptions (Fig. 2). For secondary *P. koraiensis*–*B. platyphylla* mixed forest and *Fraxinus mandshurica* plantation, June exhibited the highest concentration of  $\text{NH}_4^+$ -N while September exhibited the highest concentration of DON (Fig. 2b, d). For *P. asperata* plantation, July exhibited the highest microbial biomass-N (Fig. 2e). Although the month that showed the lowest concentration of N varied between soil N sources, these were concentrated between June and September (Fig. 2). Free amino acid-N and  $\text{NO}_3^-$ -N appeared to be the lowest in July than in other months,  $\text{NH}_4^+$ -N appeared to be the lowest mostly in August, DON appeared to be the lowest often in September but occasionally in July, and microbial biomass-N showed the lowest concentrations in all months except May (Fig. 2).

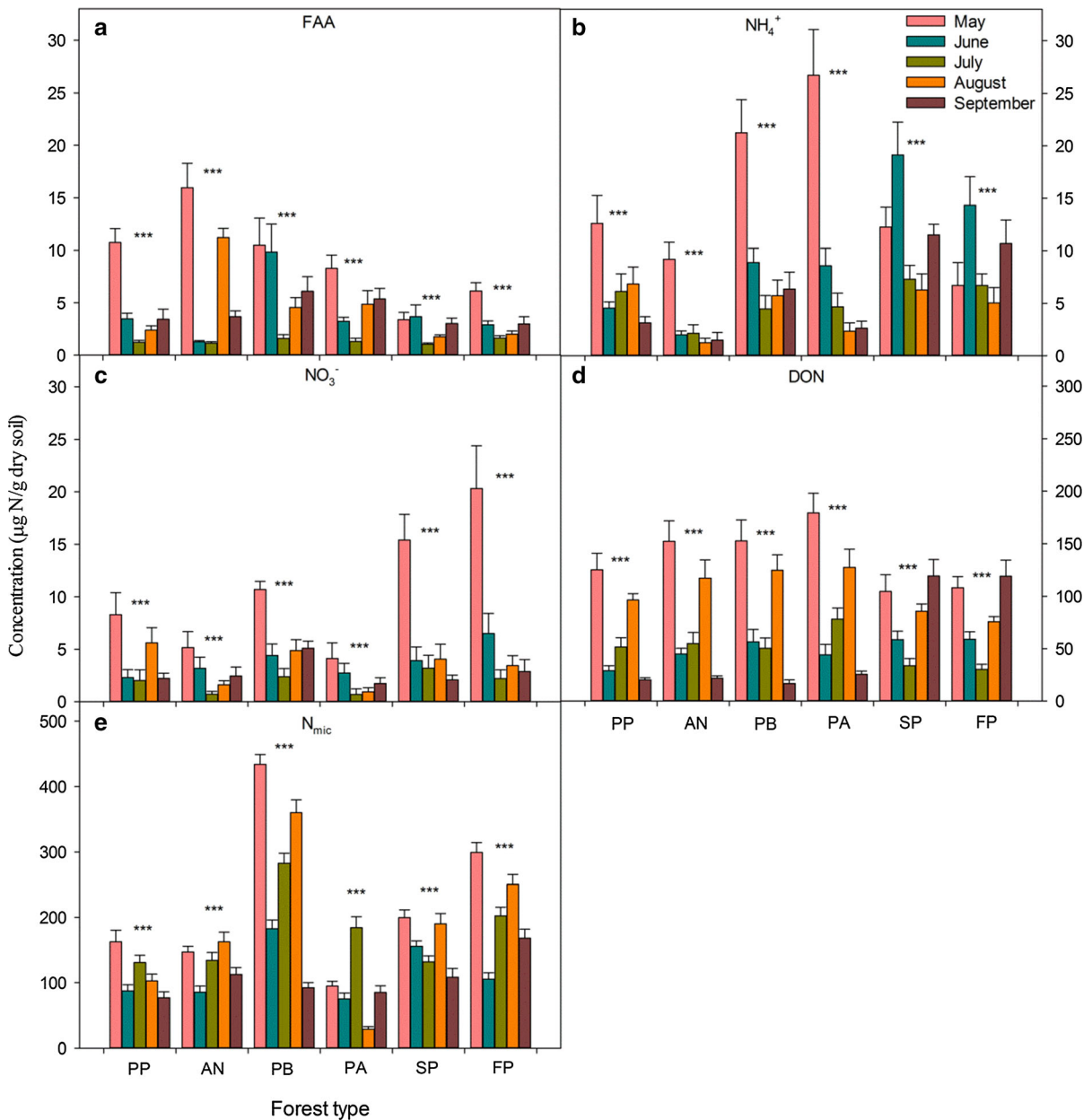
### Composition of dominant soil amino acids in temperate forests

Forest type and month also appeared to influence the composition and concentration of the dominant soil amino acids (Fig. 3; Table S1). Generally, eight dominant free amino acids were detected in the temperate forest soils, and their concentrations followed the order: arginine > histidine > serine > leucine > aspartic acid > glycine > glutamic acid > proline (Fig. 3). Except for arginine, histidine and aspartic acid, the other amino acids did not always appear to be dominant across these forest soils (Fig. 3). This was especially obvious for proline as it only appeared to be dominant in *F. mandshurica* plantation soils over the growing season, with the concentration ranging from 0.10 to 0.26  $\mu\text{g N/g}$  dry soil (Fig. 3h).

The concentrations of dominant soil amino acids also tended to be the highest in May than in other months (Fig. 3). Nevertheless, for pristine *A. nephrolepis* forest and *P. asperata* plantation, the concentrations of serine, aspartic acid and glutamic acid were the highest in August (Fig. 3c, e and g). For secondary *P. koraiensis*–*B. platyphylla* mixed forest, the concentrations of glycine and glutamic acid were the highest in September (Fig. 3f, g). The concentrations of dominant soil amino acids were the lowest mostly in July but occasionally in June and August (Fig. 3). Pristine *A. nephrolepis* forest was the only forest type that showed the lowest concentrations of many dominant soil amino acids (arginine, leucine, aspartic acid and glutamic acid) in June than in other months (Fig. 3a, d, e and g). Meanwhile, in most cases the lowest concentrations were observed in August (Histidine, aspartic acid, glutamic acid and proline) particularly in the secondary *P. koraiensis*–*B. platyphylla* mixed forest and *F. mandshurica* plantation (Fig. 3b, e, g and h).

### 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 5 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,  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , arginine, histidine and aspartic acid (Table 2; Fig. 4). Other soil

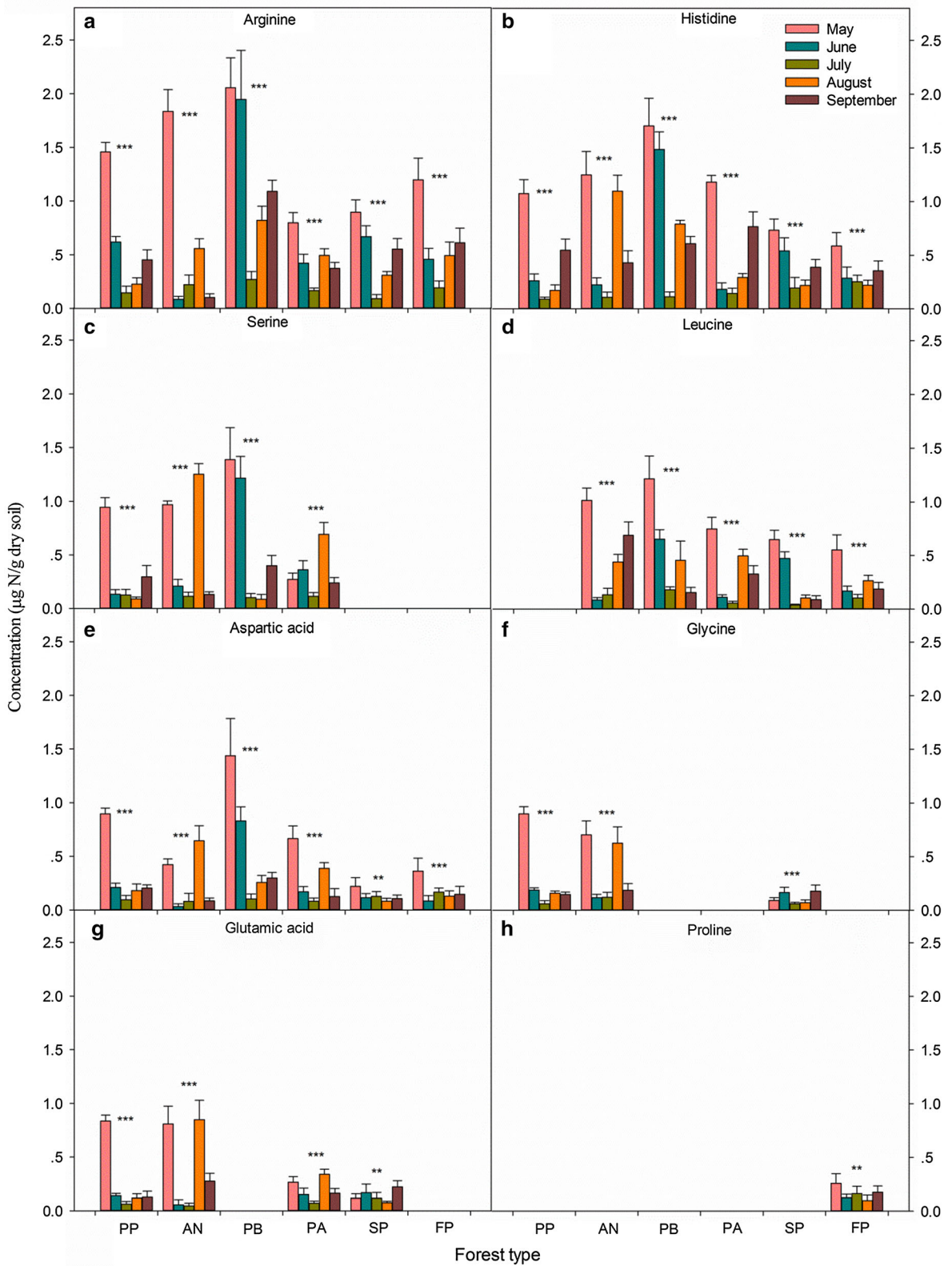


**Fig. 2** Concentrations of soil total free amino acid-N (FAA; **a**),  $\text{NH}_4^+$ -N (**b**),  $\text{NO}_3^-$ -N (**c**), dissolved organic N (DON; **d**) and microbial biomass-N ( $N_{\text{mic}}$ ; **e**) over five months. Error bars show standard deviations ( $n = 5$ ). Pristine broad-leaved *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

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  $\text{NH}_4^+$  was positively related to DON which could explain 25.6% of its variation (Fig. 4b). Soil  $\text{NO}_3^-$  and aspartic acid were both positively related to moisture and microbial biomass-





**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. Error bars show standard deviations ( $n = 5$ ). \*\* and \*\*\* indicates significant difference between months within a forest at the  $p < 0.01$  and  $0.001$  level, respectively.

N, which together could explain 31.6% and 46.0% of the variation, respectively (Fig. 4c, f).

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

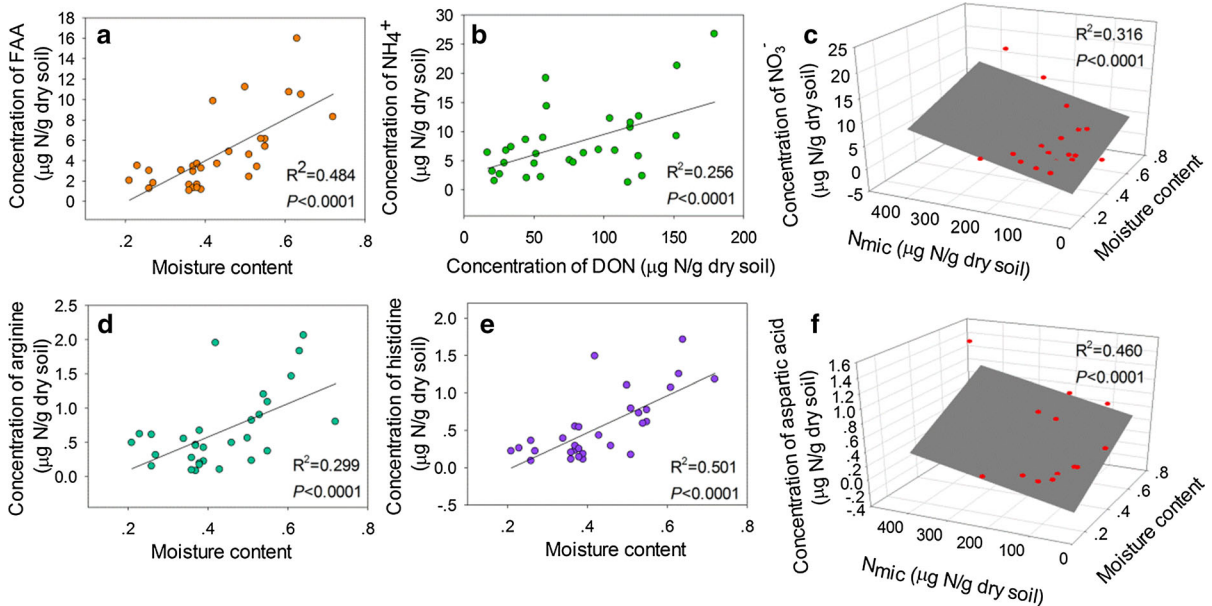
Multivariate correlations among soil amino acid-N, inorganic N, DON and microbial biomass-N were analyzed by PCA (Fig. 5). The two main components

**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 <sub>4</sub> <sup>+</sup>	DON	297.1	1	10.9	0.003
NO <sub>3</sub> <sup>-</sup>	N <sub>mic</sub> 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 <sub>mic</sub>	1.4	2	13.7	< 0.001

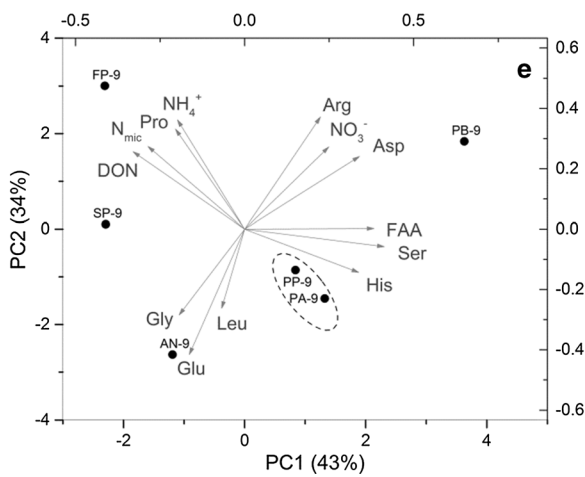
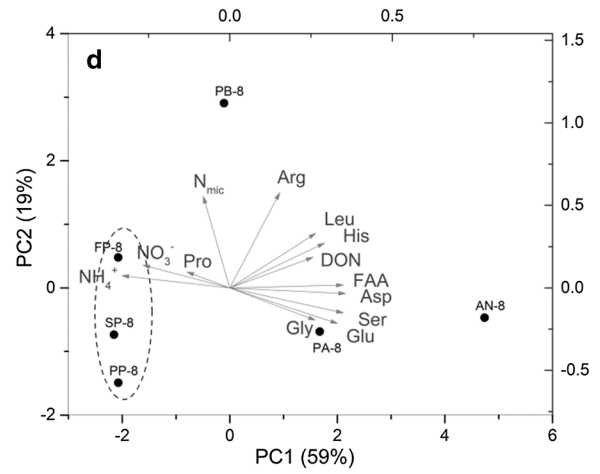
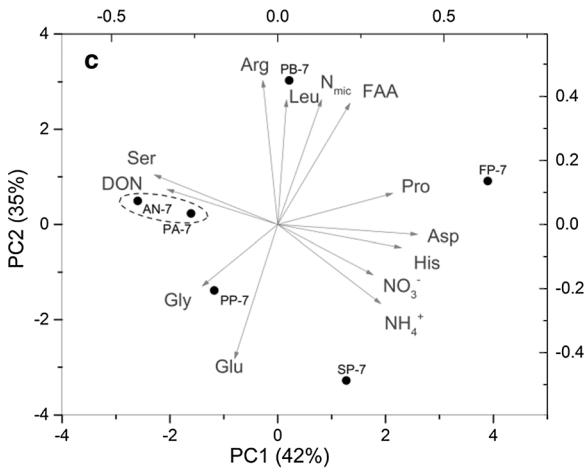
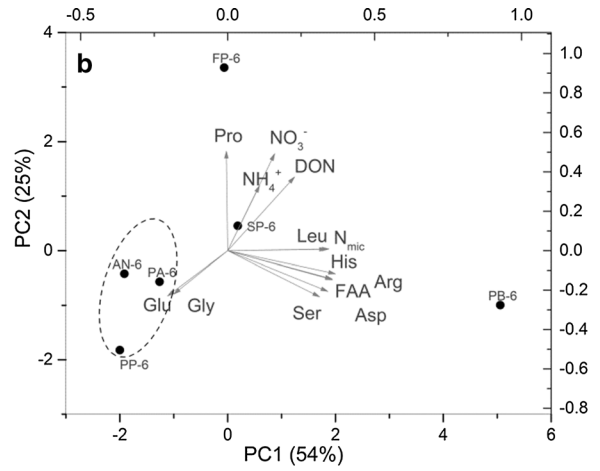
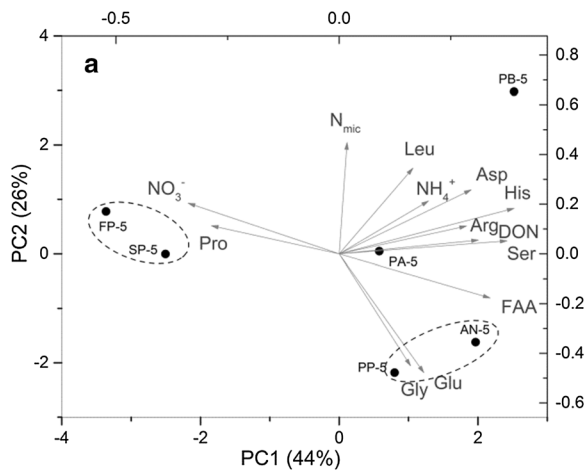
Independent variables analyzed for stepwise regression analysis includes: organic C, N<sub>mic</sub>, pH, total N, DON, moisture content and C/N ratio

FAA total free amino acids; DON dissolved organic N; N<sub>mic</sub> microbial biomass-N



**Fig. 4** Relationships between soil soluble N (total free amino acids, FAA, a; NH<sub>4</sub><sup>+</sup>, b; NO<sub>3</sub><sup>-</sup>, c; arginine, d; histidine, e; aspartic acid, f) and the related factors obtained from stepwise

regression analyses. Microbial biomass-N, N<sub>mic</sub>. 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 (His); Serine (Ser); Leucine (Leu); Aspartic acid (Asp); Glycine (Gly); Glutamic acid (Glu); Proline (Pro); total free amino acids (FAA); dissolved organic N (DON); microbial biomass-N ( $N_{mic}$ ). Numbers linked with forest types indicate corresponding months. Forest types showing similar soil N characteristics are indicated by the ovals

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 *P. koraiensis* mixed forest and *P. 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), microbial biomass-N, and DON, which is opposite to *F. mandshurica* plantation (Fig. 5e); secondary *P. koraiensis*–*B. platyphylla* mixed forest showed lower concentrations of soil free amino acid-N, histidine-N and serine-N (not dominant) (Fig. 5e); pristine *A. nephrolepis* forest showed higher concentrations of soil glutamic acid-N, glycine-N and leucine-N, whereas secondary *P. asperata*–*B. platyphylla* mixed forest showed higher concentrations of soil  $NO_3^-$ -N, arginine-N and aspartic acid-N (Fig. 5e). Second, the distribution pattern of these forest types in the PCA appeared to differ over 5 months. This could be well illustrated as the forest types that possessed similar soil N characteristics (*i.e.* the clustered forest types) changed over time (Fig. 5).

## Discussion

### Composition of dominant soil amino acids in temperate forest ecosystems

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

crucial issues, *e.g.* organic N utilization (Näsholm et al. 1998), N niche differentiation (McKane et al. 2002) and species coexistence (Ouyang et al. 2016). Here we show that arginine, histidine, serine, leucine, aspartic acid, glycine, glutamic acid and proline composed the dominant soil amino acids in the temperate forest ecosystems of northeast China (Fig. 3). This provides a basis for further research that examines the importance of dominant soil amino acid-N in plant nutrition within these forest ecosystems (Gao et al. 2019). The availability of soil amino acids is determined by the relative rates of production and consumption; when the production rate exceeds the consumption rate, its availability appears to increase and vice versa. The processes related to production and consumption involve complex biochemical mechanisms. First, soil amino acids have various sources. The breakdown of protein probably dominates (Jan et al. 2009), but dry and wet deposition (Mopper and Zika 1987), vegetation stemflow and throughfall (Michalzik and Matzner 1999), root exudation and death (Jones and Darrah 1994), microbial turnover, and faunal inputs (Hill et al. 2019a), also contribute. Second, amino acids have many sinks, including the selective use by microorganisms and plants (Endres and Mercier 2003; Lipson et al. 1999a), mineralization (Jones and Kielland 2002) and leaching (Fischer et al. 2007). Probably as a result of selective inputs or consumption, soil dominant amino acids appeared to be distinct among forest types (Fig. 3) and also seemed different to the reports from other plant ecosystems (Björk et al. 2007; Werdin-Pfisterer et al. 2012). Different forest types showed different N cycling characteristics (Fig. 5), and the variation in the composition of soil amino acids could partially reflect the variation in soil N cycling traits. This was especially prominent for *F. mandshurica* plantation, as proline was dominant only in its soils (Fig. 3h). Although we cannot explain the phenomenon, it could illustrate that the composition of soil dominant amino acids can indirectly reflect differences in soil–plant N cycling processes between plant communities. Despite these differences in factors related to N cycling and the variation in composition of the pool of amino acids, the basic amino acids, arginine and histidine, appeared to continuously dominate in soils and on average accounted for 26% of the free amino acids. This suggests that selective sorption of amino acids by the soil solid phase could also play an important role in

amino acid-N cycling in the temperate forest soils (Rothstein 2010). The soils in the studied region mostly belong to dark brown forest soils, which are rich in organic matter (Table 1) and can have a high clay content (Zhang and Song 2005). This may partially contribute to the adsorption and prevalence of basic amino acids. Additionally, aspartic acid belongs to the acidic amino acids which generally have a low sorption potential on the soil solid phase relative to basic or neutral amino acids (Gonod et al. 2006), but it also dominated in the soil amino acid pool here (Fig. 3e). Some researchers suggest that severed fine roots and mycorrhizal hyphae (Hobbie and Hobbie 2013) may lead to the prevalence of aspartic acid (Senwo and Tabatabai 1998; Werdin-Pfisterer et al. 2009). As described above, there were many potential factors that could affect the composition of soil dominant amino acids. Based on the data collected here, we cannot explain explicitly which factors induce the difference or similarity in the components of dominant soil amino acids between forest types, but this deserves further research. Additionally, soil soluble N can be primarily divided into three parts, which are mobile in soil solution, adsorbed to ion exchange sites and spatially inaccessible due to isolation in microsites (Darrouzet-Nardi and Weintraub 2014). In contrast to inorganic N (KCl extraction), deionized water as the extractant for soil amino acids, which may underestimate the concentrations of some amino acids that are prone to sorption on the soil's solid phase (e.g. histidine and arginine) (Rothstein 2010; Werdin-Pfisterer et al. 2009). Nevertheless, free amino acids may be more easily taken up by roots and microbes, representing more available N sources for organisms than the adsorbed types.

#### Seasonal dynamics of soil soluble N and potential drivers

Plant community type can significantly influence the turnover of soil C and N through the change of soil biochemical conditions, e.g. pH (Killham 1990), the activity of enzymes and the structure of the microbial community (Björk et al. 2007; Weand et al. 2010). The six forest types chosen here were comprised of different plant species, and they were characteristic of different growth stages, soil pH, microbial biomass, ratios of C to N and relative moisture (Table 1; Fig. 2e). Some of the plant communities are in the

early successional stage (secondary *P. koraiensis*–*B. platyphylla* mixed forest, secondary *P. asperata*–*B. platyphylla* mixed forest and *F. mandshurica* plantation), while the others are in a late successional stage (pristine broad-leaved *P. koraiensis* mixed forest, *P. asperata* plantation and pristine *A. nephrolepis* forest). As hypothesized, the concentration of soil soluble N was affected by plant communities (Figs. 2a, b, c, d and 3; Table S1). This is in agreement with many studies from taiga forest (Kielland et al. 2007), boreal forest (Werdin-Pfisterer et al. 2009) and tundra (Björk et al. 2007) ecosystems. As there was interaction between forest type and month on the availability of soil soluble N (Table S1), however, the distinction in the concentration of soil soluble N between plant communities varied across months (Figs. 2 and 3). For instance, the concentration of total soil amino acids in May was higher in pristine *A. nephrolepis* forest than in secondary *P. koraiensis*–*B. platyphylla* mixed forest; in August, this was reversed (Fig. 2a). In contrast to the total soil C or N pool in temperate forests, which is primarily comprised of organic matter and can be stable for years to decades (Lützow et al. 2006), inorganic N and amino acid-N can be strongly affected by many potential factors and can be easily consumed in soil solution (e.g. by roots, microbes or enzymes). Compared with season, the type of plant community showed less influence on the availability of soil soluble N in the temperate forest ecosystems of northeast China. This is different to the research from boreal and taiga forest ecosystems where the concentrations of soil amino acids have been reported to increase over the succession sequence of forest communities (Kielland et al. 2007; Werdin-Pfisterer et al. 2009). This difference suggests that the turnover characteristics of soil inorganic N or amino acid-N could vary between forest ecosystems in different biomes.

In temperate forest ecosystems, belowground biological processes are extremely influenced by the seasonal dynamics of soil temperature and moisture (Wang et al. 2006). From May to September, the air temperature and precipitation changed considerably (Fig. 1), and the characteristics of soil N cycling varied over the growing season (Fig. 5). The stronger influence of season on the availability of soil inorganic N and free amino acids than forest type, was primarily reflected in the following two aspects: (1) coefficients of variation of soil inorganic N, free amino acids and

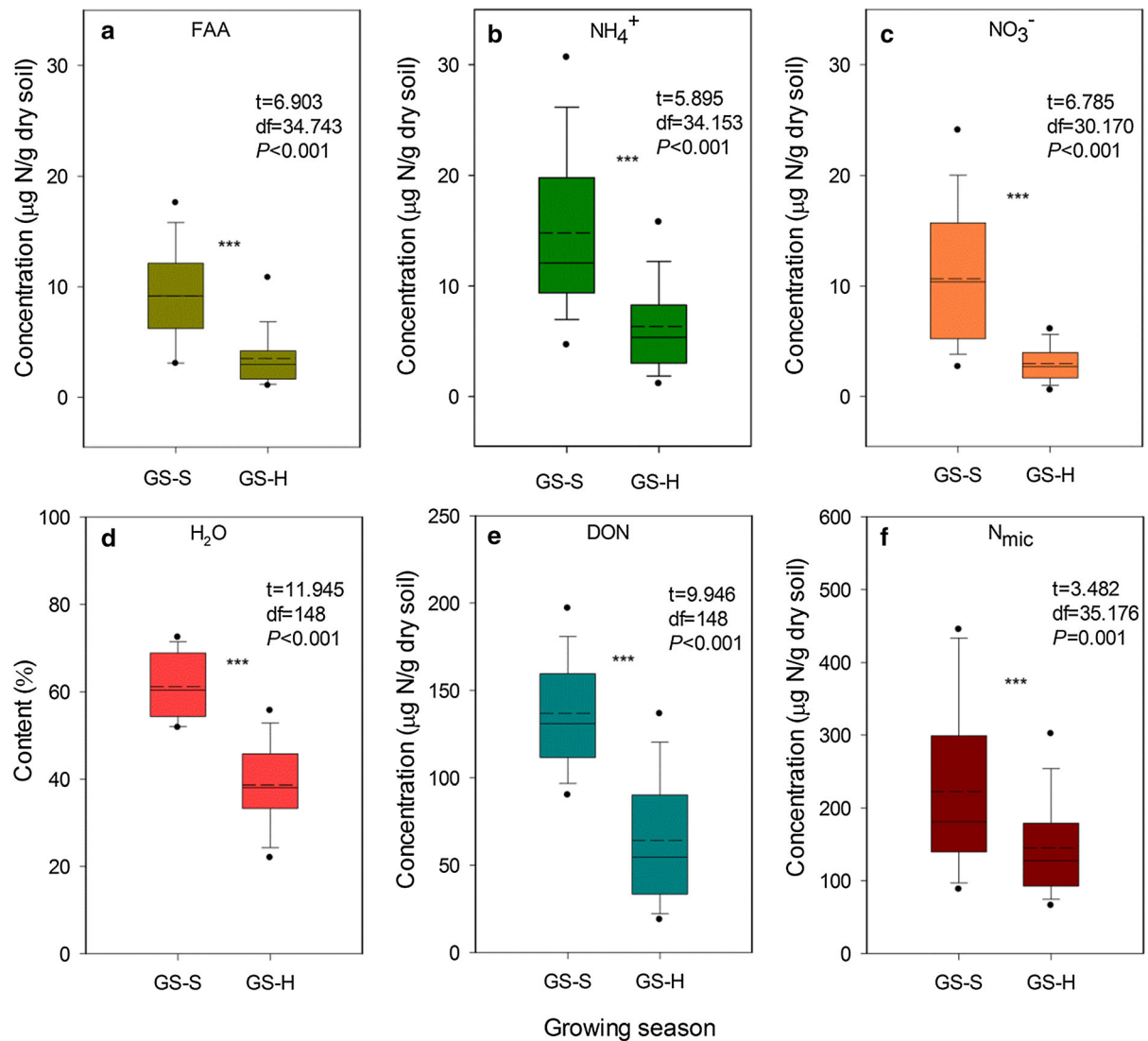
the potential drivers (moisture and DON) tended to be greater for month than for forest type, which was especially prominent for  $\text{NO}_3^-$  (Table 3). A probable explanation could be because, compared with other forms of N, soil  $\text{NO}_3^-$  was more prone to leaching induced by the seasonal change of precipitation; and (2) the concentrations of soil inorganic N and free amino acids tended to be higher at the beginning than at the height of growing season due to increased plant demand. From the onset to the height of growing season, the concentration of soil free amino acid-N,  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N decreased by 62%, 57% and 72%, respectively (Fig. 6a, b and c). Both abiotic and biotic factors could possibly lead to this phenomenon. The precipitation mostly occurred at the height of growing season (Fig. 1), which could cause the leaching of soil soluble N especially for those that are not readily adsorbed on the soil solid phase, e.g. acidic amino acids (Gonod et al. 2006) and  $\text{NO}_3^-$ . However, as rainy days or muddy periods were avoided during soil sampling, so the lower concentration of soil inorganic N, free amino acids and DON could not be directly explained by the influence of precipitation. Based on stepwise analyses, the variations of soil inorganic N and amino acids across forest types and months were potentially driven most by soil moisture, followed by microbial biomass-N and DON (avg. 7.7% of which was free amino acid-N), and the availability of inorganic N and amino acids were positively related to these potential drivers (Table 2;

Fig. 4). At the beginning of the growing season, the concentration of soil moisture, microbial biomass-N and DON was 58%, 53% and 113% higher than that at the height of growing season, respectively (Fig. 6d, e and f). In the early May of northeast China, it was shortly after the periods of soil freezing and thawing (Wang et al. 2013) which could release high amounts of water and N nutrients to the soil solution (Grogan et al. 2004; Sanders-DeMott et al. 2018). The moist conditions could strengthen the accessibility of DON sources to soil enzymes and microbes (Darrouzet-Nardi and Weintraub 2014), promoting the depolymerization and mineralization of N in soil solution. Therefore, as reported by others (Bardgett et al. 2007; Hackl et al. 2004), we also suggest that soil moisture is a very important factor for mediating the belowground processes related to soil N sources. On the other hand, both the uptake of N by soil microbes and plant roots could also play an important role in mediating the seasonal dynamics pattern of soil inorganic N and amino acids. Nevertheless, the concentration of soil microbial biomass-N also decreased by 35% from the onset to the height of growing season (Fig. 6f). As a storage pool of soil N, the turnover of microbial N is frequently very rapid (3–5 days; Kuzyakov and Xu 2013). The available soil N will ultimately flow into the plants as they are more stable N storage pool (Kuzyakov and Xu 2013). The phenology of plants is strongly influenced by seasonality, driving the dynamic uptake of soil soluble N by roots (McKane

**Table 3** Coefficients of variation of soil soluble N, moisture and microbial biomass-N across forest types and months

Soluble N	Coefficients of variation (%)				
	Forest type	Month	<i>df</i>	<i>t</i>	<i>P</i>
FAA	50 (26)	67 (22)	9	1.17	0.273
$\text{NH}_4^+$	55 (14)	72 (31)	9	1.17	0.271
$\text{NO}_3^-$	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
$N_{\text{mic}}$	44 (14)	38 (15)	9	− 0.73	0.483

FAA total free amino acids;  
DON dissolved organic N;  
 $N_{\text{mic}}$  microbial biomass-N.  
Means (standard deviations)  
are shown



**Fig. 6** Concentrations of soil total free amino acid-N (FAA; **a**),  $\text{NH}_4^+$ -N (**b**),  $\text{NO}_3^-$ -N (**c**), moisture ( $\text{H}_2\text{O}$ ; **d**), dissolved organic N (DON; **e**) and microbial biomass-N ( $\text{N}_{\text{mic}}$ ; **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

et al. 2002; Stahl et al. 2011). During the onset of the growing season, the N needed for plant growth can partially come from the remobilization of plants' stored resources, and tree growth often starts before N uptake by roots, reducing the N demand from the soils (Millard and Grelet 2010). Meanwhile, the production of roots in the typical temperate forests of these regions can increase significantly during the height of growing season (Quan et al. 2010). This can

significantly increase the uptake capacity and support the growth demand of plants for the limited N resource. Thus, the reduction in the pools of available soil N during the height of growing season in the temperate forest ecosystems most-likely results from the higher demand for N nutrient by plants (Weintraub and Schimel 2005).

## Conclusions

In the temperate forest ecosystems of northeast China, the pool size and composition of soil soluble N were influenced more strongly by season than by vegetation. Soil potential drivers (*e.g.* moisture, microbial biomass-N, DON) and the phenology of plants together drove the seasonal dynamics of soil inorganic N and amino acid-N. Arginine, histidine, serine, leucine, aspartic acid, glycine, glutamic acid and proline composed the dominant soil amino acids in these temperate forest ecosystems; regardless of vegetation and season, however, basic amino acids (arginine and histidine) dominated consistently, illustrating the potentially important role of selective sorption by the soil solid phase in mediating soil amino acid-N cycling.

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## Compliance with ethical standards

**Conflict of interest** No potential conflict of interest was reported by the authors.

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