# Organic amendment and inorganic fertilization affect soil properties and quality of Larix olgensis bareroot stock

Hongxu Wei • Chengyang Xu • Barbara J. Hawkins • Lüyi Ma • Lini Jiang

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Abstract Bareroot Changbai larch (Larix olgensis Henry.) seedlings were reared with inorganic fertilizer (nitrogen (N):phosphorus  $(P) = 1:1$ , W/W) applied at a rate of 100 (F100) or 200 kg N ha<sup>-1</sup> (F200) with (+) or without (-) chicken manure as a soil amendment (O) in north-eastern China. An unfertilized control treatment was included. Inorganic and organic fertilizer treatments tended to increase soil ammonium, nitrate, available P, total P, organic carbon content and electrical conductivity, and biomass and N concentration in seedlings. Organic amendment improved first order lateral root number, tap root length, fine root morphology (length, surface area, volume) in seedlings, while the F100 treatment increased N accumulation in needles and stems compared to the F200 treatment, on average. Most fertilizer treatments tended to increase P content in combined stems and roots, but  $F200 - O$  and  $F100 + O$  treatments diluted P in needles. Organic amendment combined with inorganic fertilizer at a rate of 100 kg N and P  $ha^{-1}$  is recommended to improve seedling growth and N reserves in woody tissues.

Keywords Seedling quality · Larix olgensis · Fine roots · Nitrogen · Phosphorus · Organic amendment

## **Introduction**

High quality planting stock will have better field performance over the long term (Davis and Jacobs [2005\)](#page-13-0). Due to ease of measurement, as well as success in predicting seedling performance after outplanting (Jacobs et al. [2005](#page-13-0)), morphology has been widely used to

H. Wei · C. Xu · L. Ma · L. Jiang Key Laboratory of Forest Silviculture and Conservation, Ministry of Education, Beijing 100083, China

H. Wei  $\cdot$  C. Xu ( $\boxtimes$ )  $\cdot$  L. Ma  $\cdot$  L. Jiang

College of Forestry, Beijing Forestry University, Beijing 100083, China e-mail: xuchybl@sina.com

evaluate seedling quality (Davis and Jacobs [2005\)](#page-13-0). The main morphological parameters used to evaluate seedling quality include seedling height, root-collar diameter, and root size (Thompson [1985;](#page-13-0) Davis and Jacobs [2005;](#page-13-0) Li et al. [2011](#page-13-0)). Root growth potential (RGP) has also been used to evaluate seedling quality and potential for new root growth (Carlson [1986\)](#page-12-0). Few reports have used fine root morphology to evaluate seeding quality though this feature may be important in determining seedling performance.

Fertilization with inorganic fertilizers during forest seedling culture is one of the most crucial factors with a positive effect on seedling quality, and thus has been well studied. Soil amendment, usually with organic matter, has also been reported to promote seedling quality (Mañas et al. [2009](#page-13-0); Davis et al. [2006](#page-13-0)). Some studies, however, found no significant effect or even negative effects of soil organic amendment on seedling morphology (Jacobs et al. [2003;](#page-13-0) Veijalainen et al. [2007](#page-13-0)). Little research of which we are aware has investigated the root response of seedlings to organic amendment applied prior to seeding (Davis et al. [2006\)](#page-13-0), and few studies have examined the interaction of inorganic and organic fertilizer on seedling growth. In regions with limited means, supplementing or replacing inorganic fertilizers with organic amendments is an attractive option.

Changbai larch (Larix olgensis), an important commercial timber species in northeastern China (Zhu et al. [2008](#page-13-0)), is valued for its rapid early growth, heavy timber, and for its characteristic strength and resistance to rot over most other softwoods. Natural regeneration is reported to be poor and the distribution of this species is decreasing (Zhu et al. [2008](#page-13-0)). The predominant types of seedlings produced by nurseries for this species are  $1 + 0$  and  $1 + 1$  bareroot stock (Li et al. [2011](#page-13-0)). The national standard in China, GB6000-1999, requires that only  $1 + 1$  seedlings be used in plantations (State Forestry Adminis-tration [2003\)](#page-13-0). However, large  $1 + 0$  seedlings of this species are still used due to their low cost (Li et al. [2011](#page-13-0)). Therefore, our objective was to find an efficient and effective fertilization regime to improve  $1 + 0$  Changbai larch seedling quality. We evaluated the effect of organic soil amendment incorporated with traditional inorganic fertilizer on soil chemical properties and seedling growth, nutrient uptake and fine root morphology. We hypothesized that inorganic fertilizer treatment and organic amendment would improve soil properties, and result in improved nutrient uptake. In addition, we hypothesized that these impacts could improve seedling growth and quality, especially root morphology.

#### Materials and methods

Study nursery and plant material

This study was conducted in Jiang Mifeng nursery (126°45'E, 43°45'N), Jilin City, Jilin Province, north-eastern China. In Jilin, annual precipitation is 650–750 mm with less than 200 mm from May to mid-June, and mean annual temperature is  $3-5^{\circ}$ C with average early growing season temperature ranging from 4 to  $9^{\circ}$ C.

Seeds of Changbai larch (*L. olgensis*) were collected by employees of Xiao Beihu Forest Station (128°28'E, 44°03'N) in a local wild stand in Hei Longjiang Province, north-eastern China. After collection, seeds were transported to the nursery and stored at  $0-4^{\circ}C$ .

Study design and fertilizer treatments

The study was conducted as a factorial design with two factors, plus a control. Inorganic fertilizer, one factor, was supplied at two rates: 200 kg N and P ha<sup>-1</sup> (F200) and <span id="page-2-0"></span>100 kg N and P ha<sup>-1</sup> (F100). Organic amendment with chicken manure (CM) was applied at two rates,  $0$  (-) and 10,000 kg (fresh weight) ha<sup>-1</sup> (+) was the second factor. Hence there were four fertilizer treatments  $(F200-, F200+, F100-, F100+)$  plus an unfertilized control (neither inorganic fertilizer nor organic amendment). There were three replicated plots for each treatment. All plots were randomly located. Treatment plots had an area of 1 m  $\times$  1 m with 0.1-0.2 m wide buffers between plots and plastic barriers inserted to a depth of 0.2 m within each buffer to eliminate lateral movement of fertilizer between plots. The N and P contents of inorganic fertilizer in the F200 treatment were close to the highest application rates for Changbai larch in operational nurseries in north-eastern China, while those in the F100 treatment were lower than the lowest application rate by at least 20%, respectively (by pre-investigation). The CM organic amendment, consisting of a local mixture of chicken feces and some soil from the southeastern part of the local nursery, had undergone natural decomposition outdoors for nearly 12 months before the experiment began. Properties of CM were determined as for the initial soil samples (Table 1). The rate of CM application, 10,000 kg ha<sup>-1</sup> (containing about 54.9 kg N ha<sup>-1</sup> and 37.8 kg P ha<sup>-1</sup>), was based on that applied to black wattle (Acacia mearnsii) (10,000 kg CM ha<sup>-1</sup>,  $\sim$ 43 kg N ha<sup>-1</sup>) (Materechera and Mkhabela [2002\)](#page-13-0), and green ash (*Fraxinus pennsylvanica*) and northern red oak (*Quercus rubra*) (1,450 kg CM ha<sup>-1</sup>,  $\sim$  48 kg N ha<sup>-1</sup> and 25.4 kg P ha<sup>-1</sup>) (Davis et al. [2006](#page-13-0)).

CM was incorporated evenly by hand raking into amended plots to a depth of 10–15 cm immediately after the seedling beds were shaped. Inorganic fertilizer was then incorporated to a depth of  $\sim$ 20 cm by further raking before sowing. Inorganic fertilizer used in this study was a 1:1 ( $W_N/W_P$ ) combination of controlled-release nitrogen (ammonium, 43-0-0, Shou Chuang Co., Beijing, China) and calcium phosphate (0-16-0, Shengda Co., Beijing, China).

Property	Soil	Chicken manure
Total N $(g \text{ kg}^{-1})$	5.71	11.93
Total P $(g \ kg^{-1})$	0.82	8.22
Total K $(g \text{ kg}^{-1})$	4.13	12.34
Total Ca $(g \text{ kg}^{-1})$	3.44	33.48
Total Mg $(g \text{ kg}^{-1})$	2.46	1.42
$NH_4^+$ -N (mg kg <sup>-1</sup> )	4.79	6.26
$NO3--N (mg kg-1)$	192.88	1.18
Available P (mg $kg^{-1}$ )	1.60	3.09
$EC_{25}$ (dS m <sup>-1</sup> )	0.10	3.51
pH(1:5)	6.51	8.23
Moisture content $(\%)$		53.98
Field water capacity (g $cm^{-3}$ )	0.35	
Organic matter (g $kg^{-1}$ )	48.40	482.32
C (g $kg^{-1}$ )	28.07	279.77
C: N	5:1	23:1

Table 1 Initial properties of the surface soil of nursery beds (0–20 cm depth) and composted chicken manure

## Seedling culture practices

Seeds were soaked in 5% potassium permanganate (W/W) solution for 24 h and stratified for 5 days at 0–4°C, then on 3 May 2009, sowed at a density of 700 seeds  $m^{-2}$  in a nursery bed. In mid June 2009, germinated seedlings were thinned to about 550 seedlings  $m^{-2}$ . Weed control was conducted manually and irrigation was performed by automatic sprayers installed along the seed beds. These activities depended on seedling growth rates and weather conditions throughout the growing season.

# Soil sampling

Prior to seeding, twelve randomly located initial soil cores (inner diameter: 4 cm, depth: 0–20 cm) were collected along the surface layer of a nursery bed. The soil texture was a sandy loam with 74.4% sand, 17.2% silt and 8.4% clay. Other specific physico-chemical properties of the nursery bed soil are presented in Table [1](#page-2-0).

On 11 October 2009, soil samples from  $0-20$ ,  $20-40$  to  $40-60$  cm depth were collected using a soil core driller. Two randomly located cores were collected per depth per plot, mixed together, passed through a 1-mm sieve, then transported to the laboratory on ice  $(0-2^{\circ}C)$  overnight for determination of ammonium-N, nitrate-N and available P contents. After determination, six fresh soil samples of the 0–20 cm layer in each treatment (2 cores  $plot^{-1} \times 3$  plots treatment<sup>-1</sup>) were mixed and air-dried (on pieces of white paper in a laboratory room with temperature of  $15-20^{\circ}$ C) to create a homogenous sample. From these bulked samples, three sub-samples were taken for determination of soil electrical conductivity (EC) and pH values and total N, P, K, Ca and Mg contents.

# Seedling sampling

On 12 October 2009, when seedlings had formed apical buds and needles had turned yellow and were about to fall, ten seedlings from each plot were selected randomly from the pool of medium-sized seedlings as defined by nursery grading standards (medium seedlings: height 6–11 cm, RCD 2–3 mm). Individual seedling height, root-collar diameter (RCD) and tap root length were measured, and number of first order lateral roots  $\geq 1$  cm length (FOLR) were counted. Then the ten seedlings per plot were bulked for measurements of biomass and N and P concentrations. These seedlings were transported to the laboratory on ice  $(0-2^{\circ}C)$ , where roots were washed free of soil.

# Seedling division and fine root morphology

The 10 bulked seedlings per plot were divided into shoot and root fractions at the cotyledon scar. Then, shoots were divided into needles and stems. Roots were divided into fine roots  $(0-2)$  mm diameter) and coarse roots  $(0-2)$  mm diameter), and fine roots were further divided into 0–1 and 1–2 mm classes for determination of root length, surface area, and volume for each diameter class through scanning by an EPSON scanner (STD  $1600+$ , EPSON Co., Japan) to gain images (.tif), which were then analysed by a WinRHIZO image analyser (Regent Instruments Inc., Quebec, Canada).

Seedling biomass and nutrient status and soil chemical analysis

Dry biomass was determined for each fraction (needles, stem, and coarse and fine roots) after oven-drying for 48 h at  $70^{\circ}$ C. Each bulked fraction was ground to pass a 1-mm sieve, then a 0.2 g sample was digested in 5 ml of  $H_2O_2-H_2SO_4$ . The digestion solution was diluted to 50 ml. Seedling total N and P concentrations were determined with 5 and 7 ml digestion solution, respectively.

For soil ammonium- and nitrate-N content determination, one 5-g fresh soil sample was extracted with 50 ml of 2 M KCl and shaken for 1 h, then analyzed using a flow injection analysis system (Lachat Instruments, Hach Co., Loveland, United States). Another 5-g fresh soil sample used for available P content measurement was extracted with 50 ml distilled water then shaken for 1 h. For dried soil samples, a 5-g sample was extracted with 25 ml distilled water for EC (Leici DDS-3.7A, Shanghai Precise Instrument Co., Shanghai, China) and pH determinations (3020 pH detector, Jenway Ltd., Dunmow, United Kingdom). Three 2-g dried soil samples were taken for measuring soil OC (Walkley and Black [1934\)](#page-13-0) and total N (Honda [1962\)](#page-13-0), P, K, Ca and Mg contents (digested in 30 ml  $HNO<sub>3</sub>$ – HClO4 [5:1, V/V]), respectively. All seedling and soil total N concentrations were determined by an automatic N analyser (UDK 152 automatic N analyser, VELP Co., Usmate (MB), Italy). Available P and total P, K, Ca and Mg concentrations in soil were determined by ICP-OES (Perkin Elmer Co., Waltham, United States).

#### Data analysis

Individual seedling height, RCD, FOLR and tap root length measurements were averaged by plot to provide a mean plot value for analysis. Plot-mean seedling biomass, nutrient (N and P) content and concentration of needles, stems, coarse roots, fine roots, and woody tissues (combined stems and roots), and fine root morphology data were calculated from the measurements of 10 bulked seedlings per plot. These plot mean values and also the plot means for measurements of soil ammonium-N, nitrate-N, and available P for each layer were analyzed with analysis of variance (ANOVA) based on the General Linear Model (GLM) procedure of SAS (9.0) (SAS Institute Inc., NC, United States), with two effects of inorganic fertilizer treatment (F) and organic amendment (O) at two levels each. All factors were considered fixed. For soil ammonium- and nitrate-N contents and available P content, soil layer (L), a fixed effect, was included in the ANOVA model, together with F and O. When treatment effects were significant, means were ranked according to Tukey's studentized range test at  $\alpha = 0.05$ . Only when the interaction of the main effects F and O showed significance, was the control treatment (Ctrl) compared with other four fertilizer treatments in a one-way ANOVA with five levels. For each parameter of fine root length, surface, and volume, differences between the two fine root classes or among different fertilizer treatments were tested using a one-way ANOVA.

As chemical properties of the 0–20 cm soil layer (total N and P and OC contents, EC and pH) were assessed on sub-samples from one bulked sample, means for each property for the five treatments were compared using simple independent *t*-tests for each comparison. Bonferroni correction was applied to adjust the significance level to account for multiple *t*-tests (n = 50) ( $\alpha$  = 0.001).

Instantaneous vector analysis was employed to facilitate interpretation of seedling growth and nutrient uptake in response to organic amendment and inorganic fertilizer treatment and in comparison of the control to fertilizer treatments. Vector shifts and their interpretations were modified from Salifu and Timmer [\(2003](#page-13-0)).

## <span id="page-5-0"></span>**Results**

## Soil properties

Inorganic fertilizer treatment and organic amendment increased total P, organic carbon content (OC) and EC of soil relative to the control, although these differences were not always significant (Table 2). The higher level of inorganic fertilizer treatment (F200) or organic amendment increased OC significantly. For example, OC of the  $F200+$  and F200- treatments, and the F100+ treatment increased by 125% ( $P \lt 0.0001$ ), 85%  $(P = 0.0005)$ , and 79%  $(P < 0.0001)$ , respectively compared to the control treatment. Organic amendment also increased EC substantially (Table 2). There was no significant effect of inorganic fertilizer or organic amendment treatments on total N content or C:N ratio, although general trends were observed. Organic amendment increased mean total soil N content in both inorganic fertilizer treatments, on average. The F200 treatment increased soil C:N ratio compared to the F100 treatment and control, on average (Table 2).

There was no significant effect of soil layer, inorganic fertilizer treatment or organic

amendment on soil ammonium content (Table [3\)](#page-6-0). Organic amendment increased soil nitrate content significantly compared to unamended soil  $(43.87 \pm 3.34)$  and  $21.58 \pm 2.28$  mg kg<sup>-1</sup>, respectively). Soil layer, inorganic fertilizer treatment, organic amendment and their interactions had significant effects on available phosphorus content (Table [3](#page-6-0)). The F200 treatment increased available P content in the 0–20 cm soil layer, and this effect was significant when organic amendment was incorporated (Fig. [1](#page-6-0)). In deeper soil layers of 20–40 and 40–60 cm, available P content generally decreased relative to the upper layer (Fig. [1](#page-6-0)). In some treatments, this decrease was dramatic. For example, available P content in the 20–40 and 40–60 cm soil layers declined by 70 ( $P = 0.0002$ ) and 77% ( $P \lt 0.0001$ ), respectively relative to that in surface soils. In the soil layer of 20–40 cm, treatments with organic amendment or a higher level of inorganic fertilizer had greater available P content. In the soil layer of 40–60 cm, there were only small differences in available P content among treatments (Fig. [1](#page-6-0)).

Treatment	Soil property								
	Total N $(g \text{ kg}^{-1})$	Total P $(g \text{ kg}^{-1})$	$OC^{\triangle}$ (g kg <sup>-1</sup> ) $C: N^{\triangle}$		EC $(dS \, m^{-1})$ pH*				
Control			$4.91 \pm 0.14$ $0.36 \pm 0.01$ a $14.57 \pm 0.97$ a $2.97 \pm 0.23$ $0.09 \pm 0.00$ a $6.10 \pm 0.05$						
$F200-$			$4.18 \pm 0.61$ 0.70 $\pm$ 0.07ab 27.02 $\pm$ 0.67bc		$6.70 \pm 0.81$ 0.19 $\pm$ 0.00b 6.28 $\pm$ 0.02				
$F200+$		$5.64 \pm 0.63$ 1.12 $\pm$ 0.19ab 32.82 $\pm$ 0.34b			$5.98 \pm 0.75$ $0.37 \pm 0.02c$ $6.84 \pm 0.05$				
$F100-$		$4.81 \pm 0.11$ $0.43 \pm 0.04$ ab $15.95 \pm 0.23$ a			$3.32 \pm 0.12$ $0.13 \pm 0.00$ $6.07 \pm 0.10$				
$F100+$		$6.46 \pm 0.20$ $0.54 \pm 0.00$ $26.06 \pm 0.56$			$4.05 \pm 0.21$ $0.22 \pm 0.00$ $6.12 \pm 0.02$				

**Table 2** Soil properties (mean  $\pm$  SE) of soil from 0 to 20 cm depth in plots from four fertilizer treatments and a control

F200 and F100 indicate inorganic fertilizer application of 200 and 100 kg N and P ha<sup>-1</sup>, respectively.  $+$  and - indicate plots with and without organic amendment, respectively. Different letters indicate a significant difference between two treatment means at  $\alpha$  < 0.008 (*Bonferroni* corrected) using independent t-tests

 $OC^{\triangle}$  organic carbon;  $C:N^{\triangle}$  ratio of organic carbon to total N content

\* Values of pH did not follow a normal distribution, therefore t-tests were not conducted

Source of variation	df	Ammonium		Nitrate		Available phosphorus	
		F	Pr > F	F	Pr > F	F	Pr > F
L	2	0.14	0.8736	1.35	0.2788	49.07	< 0.0001
F	1	0.05	0.8199	1.65	0.2111	30.10	< 0.0001
$\Omega$	1	2.32	0.1406	32.23	< 0.0001	18.67	0.0002
$L \times F$	2	1.09	0.3519	0.60	0.5551	18.51	< 0.0001
$L \times 0$	1	0.42	0.5207	0.13	0.7202	5.13	0.0329
$F \times O$	2	0.18	0.8342	1.62	0.2180	5.15	0.0137
$L \times F \times O$	2	0.62	0.5447	1.53	0.2377	7.58	0.0028

<span id="page-6-0"></span>Table 3 Analysis of variance testing effects of soil layer (L), inorganic fertilizer treatment (F), and organic amendment (O) on total ammonium, nitrate, and available phosphorus content in soil

df degrees of freedom



#### Seedling quality

The interaction of inorganic fertilizer treatment and organic amendment had no significant effect on seedling height ( $P = 0.1195$ ), RCD ( $P = 0.3085$ ), FOLR ( $P = 0.6154$ ), and tap root length ( $P = 0.8531$ ). Neither seedling height nor RCD was significantly affected by inorganic fertilizer treatment (seedling height:  $P = 0.7905$ , RCD:  $P = 0.7766$ ) or organic amendment (seedling height:  $P = 0.2330$ , RCD:  $P = 0.5198$ ). Relative to the F200 treatment, the F100 treatment increased FOLR (F100: 17.45  $\pm$  0.46, F200:  $15.22 \pm 0.52$  and tap root length (F100:  $16.22 \pm 0.71$  cm, F200:  $14.81 \pm 0.64$  cm) by 15% ( $P = 0.0010$ ) and 10% ( $P = 0.0446$ ), respectively. Organic amendment increased FOLR ( $+$ : 17.23  $\pm$  0.55,  $-$ : 15.43  $\pm$  0.60) and tap root length ( $+$ :  $16.76 \pm 0.57$  cm,  $-$ :  $14.27 \pm 0.39$  cm) by  $12\%$  ( $P = 0.0036$ ) and  $18\%$  ( $P = 0.0029$ ), respectively.

Morphological parameters of fine roots

Fine roots of 0-1 mm diameter had greater root length, surface area and volume  $(110.68 \pm 6.60 \text{ cm}, 15.43 \pm 1.35 \text{ cm}^2 \text{ and } 0.79 \pm 0.27 \text{ cm}^3 \text{, respectively})$  than roots of 1–2 mm diameter  $(18.70 \pm 1.49 \text{ cm}, 7.90 \pm 0.68 \text{ cm}^2, \text{ and } 0.27 \pm 0.03 \text{ cm}^3, \text{ respectively})$ tively)  $(P\lt 0.0001)$ . For both fine root classes, inorganic fertilizer treatments had no significant effect on fine root morphology (Fine root length:  $0-1$  mm,  $P = 0.8219$ , 1–2 mm,  $P = 0.2200$ ; surface area: 0–1 mm,  $P = 0.4794$ , 1–2 mm,  $P = 0.2392$ ; Volume: 0–1 mm,  $P = 0.2392$ , 1–2 mm,  $P = 0.6245$ ). Organic amendment increased fine root morphological parameters for both 0–1 and 1–2 mm diameter classes, but the results were not always significant. For 0–1 mm diameter roots, organic amendment increased length (+:  $125.15 \pm 7.61$  cm, -:  $96.21 \pm 7.09$  cm) and volume (+:  $0.93 \pm 0.08$  cm<sup>3</sup>, -:  $0.65 \pm 0.08$  cm<sup>3</sup>) significantly ( $P = 0.0349$  and 0.0400, respectively), and for 1–2 mm diameter roots, organic amendment increased surface area  $(+; 9.30 \pm 0.79 \text{ cm}^2, -;$ 6.51  $\pm$  0.81 cm<sup>2</sup>) significantly (*P* = 0.0399).

Biomass, and N and P contents and concentrations

The interaction of inorganic fertilizer treatment and organic amendment was significant for needle biomass ( $P = 0.0331$ ), needle N content ( $P = 0.0194$ ), and stem N concentration  $(P = 0.0252)$ . Needle biomass and N content increased with OM addition in the F200 treatment, but decreased with OM addition in the F100 treatment (Fig. [2a](#page-8-0), b). The opposition interaction was observed for stem N concentration (Fig. [2](#page-8-0)c). Although the interaction of inorganic fertilizer treatment and organic amendment was not significant for needle N concentration ( $P = 0.7067$ ) and stem N concentration ( $P = 0.0702$ ), one-way ANOVA indicated significant differences among the five treatments for these two parameters ( $P = 0.0258$  and 0.0024, respectively). N concentrations were highest in the F100+ treatment, but not significantly different from most of the other fertilized treat-ments, except F[2](#page-8-0)00+ stem N concentrations (Fig.  $2a$ ). Neither inorganic fertilizer treatment nor organic amendment affected total biomass  $(P = 0.3464$  and 0.6542, respectively). Fertilizer treatments tended to increase needle biomass, N content and N concentration compared to the control, though the results were not always significant (Fig. [2\)](#page-8-0).

The F100 treatment had higher needle N content and stem N concentration, but lower fine root N concentration and coarse root P concentration than the F200 treatment  $(P = 0.0246, 0.0008, 0.0235,$  and  $0.0245$ , respectively) (Table [4\)](#page-9-0). Organic amendment significantly increased fine root N concentration by  $9\%$  ( $P = 0.0254$ ), but decreased coarse root P concentration by 28% ( $P = 0.0029$ ).

Vector analysis

Relative to the control, fertilizer treatments appeared to alleviate N deficiency in both needles and woody tissues (Fig. [3a](#page-10-0), b). The treatments of  $F100-$  and  $F200+$  induced a P dilution in needles (Fig. [3](#page-10-0)c), but alleviated a P deficiency in woody tissues compared to the control (Fig. [3](#page-10-0)d). The  $F200-$  and  $F100+$  treatments had a small increase in needle biomass, but a greater decline of P concentration and content relative to the control (Fig. [3c](#page-10-0)). Also, vector analysis revealed a P deficiency in combined stems and roots of the control relative to the  $F100-$ ,  $F200-$ , and  $F200+$  treatments, while the  $F100+$  treatment showed a P dilution compared to the control (Fig. [3d](#page-10-0)).

<span id="page-8-0"></span>Fig. 2 Mean  $(\pm SE)$  biomass  $(top)$ , N content (*middle*) and N concentration (bottom) of needles, stems and shoots in four fertilizer treatments and a control. F200 and F100 indicate inorganic fertilizer application of 200 and 100 kg N and P  $ha^{-1}$ , respectively.  $+$  and  $-$  indicate plots with and without organic amendment, respectively. Different lower case letters within bars for needles and stems and capital letters above the bars for shoots (needles  $+$  stems) indicate significant differences according to Tukey's studentized range test at 0.05 level



## **Discussion**

#### Soil properties

As we hypothesized, most treatments with organic amendment had greater total soil N and P contents and EC, on average (Table [2](#page-5-0)). In the soils at the end of our experiment, greater OC was found in treatments with organic amendment than in treatments without organic amendment, but there were no differences among treatments in C:N ratio. Available P content was especially enriched in the upper soil layer of the F200 and organic amendment treatments (Fig. [1\)](#page-6-0). Increment of P content in soils amended by CM was found in other reports (Davis et al. [2006\)](#page-13-0). The effect of inorganic fertilizer treatment on soil properties was weaker than that of organic amendment.

Fertilizer treatment	Needles	Stem	Coarse root	Fine root	Woody tissues			
$N$ content $(mg)$								
F <sub>100</sub>	$4.41 \pm 0.18a$	$5.06 \pm 0.39$	$1.70 \pm 0.32$	$5.59 \pm 0.26$	$12.35 \pm 0.63$			
F <sub>200</sub>	$3.85 \pm 0.18$	$4.36 \pm 0.41$	$1.94 \pm 0.20$	$4.98 \pm 0.51$	$11.29 \pm 0.71$			
$\overline{\phantom{m}}$	$4.09 \pm 0.29$	$4.71 \pm 0.49$	$1.94 \pm 0.27$	$4.90 \pm 0.41$	$11.55 \pm 0.65$			
$+$	$4.16 \pm 0.11$	$4.71 \pm 0.36$	$1.70 \pm 0.27$	$5.67 \pm 0.37$	$12.08 \pm 0.76$			
<i>N</i> concentration (mg $g^{-1}$ )								
F100	$27.07 \pm 0.51$	$31.86 \pm 0.59a$	$25.30 \pm 1.40$	$28.05 \pm 0.45$	$29.12 \pm 0.28$			
F <sub>200</sub>	$26.58 \pm 0.19$	$28.14 \pm 0.75b$	$22.61 \pm 0.34$	$30.59 \pm 1.06a$	$27.93 \pm 0.45$			
	$26.02 \pm 0.31$	$30.57 \pm 0.70$	$25.27 \pm 1.23$	$28.07 \pm 0.91B$	$28.42 \pm 0.55$			
$+$	$27.03 \pm 0.45$	$29.43 \pm 1.30$	$22.63 \pm 0.76$	$30.57 \pm 0.72$ A	$28.63 \pm 0.34$			
<i>P</i> content (mg)								
F <sub>100</sub>	$0.23 \pm 0.02a$	$0.30 \pm 0.03$	$0.10 \pm 0.02$	$0.41 \pm 0.04$	$0.81 \pm 0.07$			
F <sub>200</sub>	$0.16 \pm 0.01$	$0.27 \pm 0.03$	$0.16 \pm 0.01$	$0.35 \pm 0.04$	$0.77 \pm 0.06$			
$\overline{\phantom{m}}$	$0.19 \pm 0.02$	$0.28 \pm 0.03$	$0.15 \pm 0.02$	$0.08 \pm 0.03$	$0.78 \pm 0.05$			
$+$	$0.18 \pm 0.01$	$0.29 \pm 0.03$	$0.11 \pm 0.02$	$0.11 \pm 0.04$	$0.80 \pm 0.08$			
<i>P</i> concentration (mg $g^{-1}$ )								
F <sub>100</sub>	$1.20 \pm 0.08$	$1.88 \pm 0.07$	$1.56 \pm 0.10$	$2.05 \pm 0.19$	$1.89 \pm 0.09$			
F <sub>200</sub>	$1.24 \pm 0.04$	$1.70 \pm 0.05$	$1.83 \pm 0.12$	$2.15 \pm 0.12$	$1.91 \pm 0.05$			
$\overline{\phantom{m}}$	$1.26 \pm 0.06$	$1.79 \pm 0.08$	$1.49 \pm 0.10B$	$2.05 \pm 0.15$	$1.92 \pm 0.05$			
$+$	$1.18 \pm 0.06$	$1.79 \pm 0.07$	$1.90 \pm 0.07$ A	$2.15 \pm 0.17$	$1.87 \pm 0.08$			

<span id="page-9-0"></span>Table 4 N and P contents and concentrations of needles, stem, coarse root, fine root, and woody tissues in organic amendment and inorganic fertilizer treatments

Woody tissues, combined stem and root

F100 and F200 indicate inorganic fertilizer applied at 100 and 200 kg N ha<sup>-1</sup>, respectively.  $+$  and  $$ indicate treatment with and without organic amendment, respectively

Different lower case and capital letters indicate significant differences at 0.05 level for inorganic fertilizer treatment and organic amendment treatment, respectively

The substantial rise in EC values with addition of CM in our study concurred with the report of Agbede et al. ([2008\)](#page-12-0). K and Ca content of the CM used in our study may have contributed to the higher values of EC (Table [1](#page-2-0)). Cation exchange capacity or cation release may be raised in treatments with CM (Materechera and Mkhabela [2002;](#page-13-0) Davis et al. [2006\)](#page-13-0) through the effects of high EC (Table [2](#page-5-0)). High EC may also help to hold P anions in the  $0-20$  cm soil layer (Fig. [1\)](#page-6-0). Although there is no published information on the effects of EC on L. olgensis seedlings, Picea amriana and Picea glauca seedlings were found to growth well at an EC up to 2.5 dS  $m^{-1}$ , but an EC of 4.0 dSm<sup>-1</sup> was fatal (Phillion and Bunting [1983\)](#page-13-0).

A hypo-buffering capacity of CM to raise pH of acidic soils has been observed (Dikinya and Mufwanzala [2010](#page-13-0)). In our study, nursery soils were slightly acidic (Table [1](#page-2-0)), and CM tended to increase soil pH (Table [2](#page-5-0)).

#### Seedling morphology

Unlike other studies (Mullin and Bowdery [1977](#page-13-0); Davis et al. [2006\)](#page-13-0), we failed to find significant responses of seedling height and RCD to either inorganic fertilizer or organic

<span id="page-10-0"></span>

Fig. 3 Vector analysis of the comparison of fertilizer treatments to the control on biomass, N (top) and P (bottom) contents and concentrations of needles (left) and woody tissues (right). Shift A nutrient dilution in the treatment relative to control, Shift C nutrient deficiency in the control, Shift G depletion in the treatment

amendment treatments. We surmise high germination density in our study ( $\sim$  550 seedlings  $m^{-2}$ ) may affect seedling height and RCD, whereas seedling densities in other studies on green ash and red oak ( $\sim$  75 seedlings m<sup>-2</sup>) (Davis et al. [2006](#page-13-0)) and white spruce seedlings (P. glauca [Moench] Voss) ( $\sim$  161 or 332 seedlings m<sup>-2</sup>) (Mullin and Bowdery [1977\)](#page-13-0) were lower.

An important component of seedling quality is the ability to produce roots quickly (Mañas et al. [2009\)](#page-13-0). Larger root systems before outplanting could result in more new root growth. Carlson ([1986\)](#page-12-0) found loblolly pine (Pinus taeda) seedlings with large initial root volume had better RGP. In our study, a higher level of inorganic fertilizer impacted seedling quality by reducing production of FOLR, and tap root length. Addition of organic amendment increased FOLR, tap root length, and fine root growth, but inorganic fertilizer did not affect fine root morphology. Superior improvement of root morphology by organic amendment relative to inorganic fertilizer could be attributed to soil resource heterogeneity which can affect the competitive relationships of fine roots and influence fine root initiation (Gross et al. [1993](#page-13-0)). As a result of organic amendment, heterogeneity in resource

distribution is likely to arise and the subsequent microbial decomposition of both simple and complex organic materials will release organic and inorganic N for plant uptake. The spatial and temporal release of these nutrients will be more complex than when inorganic nutrients are applied directly.

Also, nitrate and phosphate availabilities in amended soil could improve root morphology. High nitrate availability has been shown to decrease primary root length relative to shoot biomass but not affect lateral root density across a range of uniform nitrate supplies (Linkohr et al. [2002\)](#page-13-0). When nitrate supply is patchy, however, lateral root density has been shown to increase in high nitrate patches (Linkohr et al. [2002](#page-13-0)). Also, Linkohr et al. ([2002\)](#page-13-0) reported that uniform low phosphate supply decreased primary root length and increased lateral root density, but high phosphate patches had no effect. Due to both an increase of FOLR and taproot length with organic amendment compared to inorganic fertilizer, we surmise that FOLR increased mainly in soil patches with abundant nitrate in amended soil. Taproot length may have been mainly stimulated by high available P content in treatments with organic amendment (Fig. [1](#page-6-0)).

Finally, the distribution of roots is influenced by soil physical features (Gebauer et al. [2011\)](#page-13-0), and organic amendment may facilitate root penetration by improving porosity (Fares et al. [2008\)](#page-13-0) and stabilizing soil structure (Ferreras et al. [2006\)](#page-13-0).

## Biomass and N and P uptake and allocation

Total biomass of whole seedlings was not affected by fertilizer treatments. This may be because all sampled seedlings were from one size category. However, the F100- treatment without organic amendment increased needle biomass and N content, relative to the control, while the same was true for the  $F200+$  treatment with organic amendment (Fig. [2a](#page-8-0), b). Vector analysis indicated that P content was less in the  $F200-$  and  $F100+$ treatments than in other treatments, which may have contributed to the poor performance of seedlings in the  $F200-$  and  $F100+$  treatments. Higher stem N concentration in the  $F100-$  and  $F100+$  treatments compared to the  $F200+$  $F200+$  $F200+$  treatment (Fig. 2c) indicated that organic amendment of soils supplied with inorganic fertilizer at a high rate of 200 kg N and P ha<sup>-1</sup> diluted N in needles and stems. Significantly higher N concentration and content in needles and stems in the  $F100+$  treatment relative to the control (Fig. [2](#page-8-0)c) indicated effective N uptake in seedlings in this treatment.

All four inorganic and organic fertilization treatments tended to improve seedling biomass and N uptake relative to the control (Fig. [3](#page-10-0)a, b). The treatments of F100- and  $F200+$  induced a P dilution in needles (Fig. [3](#page-10-0)c), but alleviated a P deficiency in woody tissues compared to the control (Fig. [3d](#page-10-0)). It seemed that P in these two treatments was mainly allocated to woody tissues, while biomass accumulated in needles (Fig. [2a](#page-8-0)) without equivalent P allocation. P depletion in needles of the F200- treatment relative to the control (Fig. [3](#page-10-0)c) may have contributed to the improved P status of woody tissues (Fig. [3](#page-10-0)d). Both needles and woody tissues, however, showed  $P$  dilution in the  $F100+$  treatment relative to the control (Fig. [3c](#page-10-0), d). Based on these results, we surmise that organic amendment tended to reduce P uptake by  $L$ . *olgensis* seedlings, especially when P was supplied at a low rate of 100 kg  $ha^{-1}$ . There are few reports of the interaction of organic amendment and inorganic fertilization on tree seedlings, but some studies show that the effect of organic amendment on P uptake is species-specific, or not stable for tree seedlings. Coleman et al. ([1987\)](#page-13-0) reported that different compost depths resulted in significant P uptake in Douglas-fir (Pseudotsuga menziesii) and noble fir seedlings (Abies procera), but not in ponderosa pine (*Pinus ponderosa*) seedlings. Veijalainen et al. ([2007\)](#page-13-0) found that, for <span id="page-12-0"></span>Norway spruce *(Picea abies)* seedlings, although needle P concentration in seedlings cultured in substrates with 50% compost were higher that seedlings supplied with 25% or no compost, results were not significantly different between the 50 and 100% compost treatments.

Though we have shown advantages of CM for seedling growth and nutrient uptake, CM was always applied in conjunction with inorganic fertilizer. A stronger design would have included a CM treatment with no inorganic fertilizer to test the effect of CM addition alone on seedling morphology. In addition, all results are only based—on a single rate of applied CM. Seedling responses to different rates of CM addition may vary with species. Davis et al. [\(2006](#page-13-0)) found that when rates of applied CM were raised from 725 to 2,900 kg ha<sup>-1</sup>, seedling height of red oak decreased but that of green ash seedling increased. In crop species, the higher the CM application rate, the higher the tissue N concentration of maize (Materechera and Salagae [2002\)](#page-13-0). For soybean, however, a higher <sup>15</sup>N recovery was seen in a lower CM application rate (Tagoe et al. [2008](#page-13-0)). Tagoe et al. [\(2008](#page-13-0)) also found that <sup>15</sup>N recovery from dried CM was greater than from carbonized CM. Additionally, CM application time, composting methods and composing materials may influence seedling responses.

# Conclusion

For bareroot L. olgensis seedlings, organic amendment with CM, a readily available and inexpensive organic fertilizer, applied at the rate of 10,000 kg  $ha^{-1}$  was recommended for its improvement of soil properties, FOLR, tap root length, fine root morphology, biomass accumulation and N uptake. With respect to traditional inorganic fertilization protocols for north-eastern China, an application rate of 200 kg N ha<sup>-1</sup> resulted in a decline of needle biomass and N content and stem N concentration and an excess of P, and failed to improve seedling quality relative to 100 kg N  $ha^{-1}$ . Interaction of inorganic fertilizer treatment and organic amendment could increase shoot growth and N uptake compared to unfertilized seedlings, but fertilizer treatments induced a P dilution in needles. Also, the treatment of  $100 \text{ kg}$  N ha<sup>-1</sup> with organic amendment diluted P in stems and roots, combined.

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