

Foliar N:P Stoichiometry in *Aralia elata* Distributed on Different Slope Degrees

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Abstract

Plant nitrogen (N) to phosphorus (P) stoichiometry is of essentially ecological meaning to non-wood forest production (NWFP) plant community in the temperate forest ecosystem. In this study, natural *Aralia elata* (Miq.) Seem. communities in montane areas of southern Heilongjiang Province, Northeast China were investigated for plant density, vegetative growth, and soil and leaf parameters on slopes in 5°, 9°, and 14° degrees. We found that individual height was greater in sites on 5° (1.6 m) and 9°-degree slopes (1.9 m) than on the 14°-degree slope (0.8 m), but soil available P content was highest on the steepest slope (5.5, 4.0, and 16 mg kg⁻¹, respectively). The foliar N:P ratio ranged 6-13. Nitrogen availability tended to promote community density and individual growth; while P availability tended to depress density but promote foliar biomass accumulation. By the diagnosis of plant nutritional monogram, the better growth and high foliar N:P ratio of about 13:1 in *A. elata* individuals on 9°-degree slope were generated by both deficiency-driving N uptake and excessive P depletion. We recommend the 9°-degree slope to develop *A. elata* community, which can be fed by higher N availability if higher density was achieved.

Keywords: cilongya; mountain vegetable; nutrient state; under-canopy resource; wild vegetable

Introduction

Nitrogen (N) and phosphorus (P) are two most important macro-nutrients limiting wild plant growth in terrestrial ecosystems. Plant N:P stoichiometry is strongly influenced by nutrient availability, and can effectively indicate the changes in the N and P cycles (Mao *et al.*, 2016). Since Koerselman and Meuleman (1996) put forward the critical value of vegetation N:P ratio as a tool of diagnosed nutrient state on a community level for wetland plants, arguments continued to prove or revise this concept in different ecosystems (Tessier and Raynal, 2003; Drenovsky and Richards, 2004; Li *et al.*, 2011; Mo *et al.*, 2015; Zhang *et al.*, 2015). Global change drivers, such as elevated [CO₂], N and P addition, drought, and species invasion, have been found to modify N and P cycles in several ecosystems (Mo *et al.*, 2015; Jing *et al.*, 2017; Sardans *et al.*, 2017), which increased the uncertainty for the change of N:P stoichiometry. In the forest ecosystem, the N and P limitations have been indicated by plant N:P ratio for several times in dominant trees or overstory plants (Barron-Gafford *et al.*, 2003; Weand *et al.*, 2010; Mo., 2015; Cao

and Chen, 2017; Jing *et al.*, 2017). Less is known about N:P stoichiometry and nutrient status of understory vegetation (Tessier and Raynal, 2003; Sitters *et al.*, 2013). Due to the interplay between adult trees and understory plants, such as the retention of N deposition by crown (Tessier and Raynal, 2003) and competition for soil sources (Lewis and Tanner, 2000; Sitters *et al.*, 2013), there are even more uncertainties to indicate N and P states in understory community by N:P stoichiometry than that in the dominant trees.

The unsustainable development of timber production and the depletion of the timber industry in a global economy context have together contributed to the demand for the development of natural sources in remote rural areas. Non-wood forest products (NWFPs) are defined as goods from biological origin other than timber production which are derived from forests, wooded lands and trees outside forests (FAO, 1999). These products are produced mainly from understory plant community, including juvenile trees, herbs, fungi, or animals (Abraham *et al.*, 2015). Many NWFPs have significant values in commerce and economy on local and national level. Worldwide, the number of reported non-wood plant species with commercial importance was estimated to be 4000 to 6000 (Secretariat of

the Convention on Biological Diversity, 2001). However, many wild plant species remain to be unknown for the potential to be developed as NWFPs' sources.

Aralia spp. belongs to the Araliaceae (Ginseng family), which is widely taken as a wild vegetable in China (Kang *et al.*, 2014), the Russian Far East, Japan, Korea and northeastern America (Sun *et al.*, 2017). *A. elata* is generally taken as an example of a medicinal plant used in China, Korean, and Japanese traditional medicine (Shikov *et al.*, 2016). *A. elata* (Miq.) Seem. ('Cilongya' or the Japanese angelica tree) is known to have various pharmacological activities for the treatment of arrhythmia, tumor, inflammatory, hypoglycemic, fatigue, and diabetes (summarized by Kim *et al.*, 2017; Sun *et al.*, 2017). *A. elata* grows as undergrowth with distribution from the ground to the altitude of 1,500 m above the sea level (Kang *et al.*, 2006). Although *A. elata* has been successfully produced through transgenic regeneration from transformed roots (Kang *et al.*, 2006), the plantlets still need to be cultured under the condition mostly being similar to the ambient of natural distribution. *A. elata* can regenerate after forest-fire and might account for a great proportion of total recruitment of undergrowth in sloping fields (Goto *et al.*, 1996). *A. elata* is one of the major competitors to pine and oak in the secondary forest (Higo *et al.*, 1995). Botanical bioassay has revealed that the leaf development in *A. elata* is related to the partial-shoot concept (Charlton, 2009). However, the information about the N:P stoichiometry in this species is rarely documented. This knowledge gap restricts the availability to the ecological culture and the cultivated evidence.

China is a very important arena for studying NWFPs due to high diversity of mountain plant species, the long-term tradition of edible wild-plants, and the socio-economic status of the population towards mountain plants (Kang *et al.*, 2014). NWFPs have been investigated in several provincial regions of China, such as Hunan (Zou *et al.*, 2010), Inner Mongolia (Wujisguleng and Khasbagen, 2010), Sichuan (Huber *et al.*, 2010), and Tibet (Kang *et al.*, 2014), Yunnan (Xu *et al.*, 2004; Ju *et al.*, 2013). Most of these regions locate in the sub-tropical area around the latitude of 30° and some locate in the temperate area between latitudes of 35° and 40°. Northeast China contains three provinces of Heilongjiang, Jilin, and Liaoning and some eastern regions of Inner Mongolia which all locate in latitudes over 40°. *A. elata* is one of the most popular mountain vegetables and ecological tonics in many regions of Northeast China as in Russia, Korea, and Japan. Nevertheless, the knowledge about *A. elata* in Northeast China is much scarcer than that in other neighbor countries (Shikov *et al.*, 2016), although Northeast China is an important headstream to export vegetative productions from *A. elata* to Southern Korea and Japan. Therefore, to clarify the vegetable growth of *A. elata* at the community scale in Northeast China is important for the whole industry of this NWFP species globally.

In this study, wild *A. elata* communities were studied to identify N and P states therein by detecting foliar N:P stoichiometry in *A. elata* plants in Northeast China. Given that *A. elata* communities usually distribute in sloping fields

(Goto *et al.*, 1996), both its density and vegetative growth were compared among three communities in fields on three slope degrees. We hypothesized that: (i) the density and vegetative growth of *A. elata* was better on higher-slope-degree fields because of less competition from dominant trees, and (ii) the *A. elata* community was co-limited by N and P which can be indicated by the foliar ratio of N:P concentrations.

Materials and Methods

Study site

This study was conducted in montane areas (45° 27' - 46° 04' N, 129° 00' - 129° 40' E) at southern Heilongjiang Province, Northeast China. The study area belongs to the temperate continental monsoon climate zone, where annual averaged temperature is 2.2 °C with the frost-free period to be 90~120 d. This area was deforested by 1870s and has been naturally regenerating with no loggings since 1998.

The *A. elata* communities are widely distributed in our study area on slopes in degrees of 5°, 9°, and 14°. The 9° degree slope site lay at the waist (elevation: 360 m) of the south-west side of the hill managed by the Baomazhuang Forestry Station. Sites on 5° and 14° slope degrees lay in the area governed by Wanbaoshan Forestry Station. The 5° degree slope site lies in a valley at the foot (elevation: 151 m) of the west side of a small hill, and the 14° degree slope site (elevation: 184 m) lies at the south-west side of the same hill. *A. elata* communities on each of the three slope-degrees naturally distributed in woodlands with the area of at least 50-thousand ha.

Stand establishment

After a reconnaissance of all three areas with different slopes, three 100 m² (10 m × 10 m) sites were randomly positioned for *A. elata* communities in August 2017. The 5°-slope sites were dominated by *Quercus mongolica* Fisch. ex Ledeb (8-10 m height, 12-16 cm diameter at breast height [dbh], 400 stems ha⁻¹ density). The 9°-slope sites were dominated by *Betula platyphylla* Suk. (10-16 m height, 12-26 cm dbh, 500 stems ha⁻¹ density) and *Pinus koraiensis* Sieb. et Zucc. (6-8 m height, 4-12 cm dbh, 900 stems ha⁻¹ density). The 14°-slope sites were dominated by *Populus davidiana* (9-17 m height, 18-19 cm dbh, 3300 stems ha⁻¹ density).

Site survey and sampling

The sites were set to survey the growth and population characteristics of *A. elata*. The 9°-slope sites were established and surveyed on August 28, 2017 and the 5°- and 14°-slope sites were investigated in the following day. The density, height and root-collar diameter (RCD) were firstly measured for all *A. elata* plants in each site. Thereafter, three 1m × 1m quadrats were positioned across the diagonal of each site as the sub-plots for investigation on leaf covering ratio and for leaves and soil sampling. In each sub-plot, the leaf-covering ratio was estimated by the covering area divided by the one square meter and soils were collected at the depth of 0-10 cm from two randomly chosen soil cores. The two soil samples from the two layers were mixed as the bulked sample for the sub-plot and three mixed soil samples

were labelled for the average measuring unit for the stand. All leaf and soil samples were transported on ice (0~4 °C) to the laboratory in 48 h.

Sample treatment

In the laboratory, fresh leaf samples were freezer-stored at the temperature of -10 °C until used for the determination of enzyme activity. Both fresh and dry weight were all measured for collected leaves so as to calculate the temporary water content (%) and subsequently the dry weight of fresh leaves used for enzyme assay. Biomass was measured after oven-dried at 70 °C for 3 days. Soil samples were air-dried for 7 days. Dried samples were ground into powder, passed 1-mm sieve, and used for determination.

The assay for the green colour degree in leaves

The green colour degree is an easily-determined parameter reflecting the synthesized state of photosynthesis, health, and growth vigor (Rabara *et al.*, 2017). Four leaves were randomly chosen from the pool for one sub-plot and scanned for the 300 dpi image (HP Deskjet 1510 scanner, HP Inc., Palo Alto, CA, USA). The leaf image (.gif format) was opened in the Photoshop CS V 8.0 (Adobe, San Jose, CA, USA) and removed for all background colours. The four leaf features were targeted in one marquee and analysed as an intact histogram, wherein the unfolded panel would indicate the average value of colour index for the channel in the sole green.

The assay for glutamine synthetase (GS) enzyme activity

The GS activity was assayed by a method modified from the procedure by Serapiglia *et al.* (2008). Leaf samples in the weight of 0.5 g were homogenized in 5 ml extraction buffer (3.059 g Tris, 0.249 g MgSO₄•7H₂O, 0.309 g dithiothreitol, and 68.5 g sucroses dissolved in 500 ml deionized water with pH 8.0 by 0.05 mM HCl) at 6000 rpm for 20 min. The homogenate was subsequently continued to be centrifuged (14000 rpm, 4 °C, 10 min) and used for GS assay. The 0.7 ml homogenate was added to 6-ml reaction B (6.118 g Tris, 9.959 g MgSO₄•7H₂O, 1.726 g monosodium glutamate, 1.211 g cysteine, 0.192 g EGTA, pH 7.4, 500 ml) with the solution of 0.7 ml ATP (40 mM). The reaction mixture was incubated at 37 °C for 30 min and stopped by adding 1.0 ml of ferric chloride reagent (3.317 g trichloroacetic acid, 10.102 g FeCl₃•6H₂O, 5 ml sulfuric acid, 100 ml). The absorbance of the product glutamyl-γ-hydroxamate was measured at 540 nm. Protein concentration was determined by the procedure of coomassie brilliant blue G-250 (Li *et al.*, 2000). Briefly, the 0.5 g fresh sample was ground, centrifuged at 10000 rpm for 10 min, measured using 1.0 ml supernatant for protein concentration at 595 nm by adding 5 ml coomassie brilliant blue G-250.

The assay for acid phosphatase (ACP) enzyme activity

The GS activity was determined by a modification of the assay described by Kolari and Sarjala (1995). The 0.1 g sample was ground in liquid N with 10 ml of 0.05 M Tris-HCl buffer, containing 1% PVP-10 and 1 mM dithioerythritol, pH 7.4. Ground sample was centrifuged at 10000 g for 10 min. The supernatant was added to 10 ml

mixture containing 0.2 mM CH₃COONa, 0.6 mM p-nitrophenylphosphate (p-NPP), pH 5.8. The reaction was incubated in dark at 25 °C for 30 min and ended by adding 1 ml 6 M NaOH. Absorbance of the reaction mixture was measured spectrophotometrically at 405 nm.

The determination of soluble sugars and starch

Soluble sugars (glucose, fructose and sucrose) and starch concentrations were determined by the colorimetric method (Wei *et al.*, 2014) using a spectrophotometer at 490 nm (UV-Visible 8453, Agilent Technologies Inc., Santa Clara, CA, USA). Briefly, 0.5-g sample was added to 50 ml of distilled water, steamed by the high pressure for two hours and determined for the concentration of soluble sugars; thereafter the residual was washed by distilled water, oven-dried, added to hydrochloric acid, extracted in boiling water bath for eight hours, added with sodium hydroxide solution and determined for starch concentration.

The determination of N and P concentrations

Total N and P concentrations were determined with the modification of the method described by Li *et al.* (2017). The 0.2 g sample was digested in 5 ml mixture of hydrogen peroxide and sulfuric acid. The digestion solution was diluted to 50 ml with deionized water. Total N concentration was determined using the Kjeldahl method. Total P concentration was determined by the Molybdenum-anticolorimetry method (Bao, 2000).

Soil determination

Soil properties (ammonium-N, nitrate-N, available P and pH) were determined with the method described by Wei *et al.* (2012). For soil ammonium- and nitrate-N concentration determination, one 5 g soil sample was extracted with 50 ml of 2 M KCl and shaken for 1 h. For available P determination, another 5 g soil sample was extracted with 50 ml distilled water and shaken for 1 h. The solution was analyzed using a flow injection analysis system (Lachat Instruments, Hach CO., Loveland, United States). Soil pH was measured (3020 pH detector, Jenway Ltd., Dunmow, United Kingdom) after 5 g sample was extracted with 25 ml distilled water and shaken for 3 h.

Statistical analysis

Firstly, all data about community, growth, leaf analysis and soil properties for *A. elata* were compared among sites in different slopes by the analysis of variance (ANOVA). The parameters about plant growth and density were measured at the site level ($n = 3$), but leaf and soil parameters were measured at the sub-plot level ($n = 9$). To eliminate the possibility of the first-type error, data from leaf and soil parameters were firstly averaged with three sub-plots as the site value, which was subsequently used in ANOVA. Data were analysed using SAS software (SAS Institute Inc., NC, USA). When significant effect of slope was detected, results were compared and arranged according to the Duncan test at the 0.05 level using the GLM procedure. To better describe multivariable relationships among variables the principle component analysis (PCA) was employed using the PINCOMP procedure. Data were subsequently analysed by either

Pearson or Spearman correlations to detect the relationship between determinative parameters and vegetative growth using the CORR procedure. Furthermore, to illustrate the formation of leaf N:P ratios in relation to nutrient state among slope degrees, the N:P ratio was analysed by a binary diagram. Finally, vector analysis was employed to clarify the nutritional state for N and P in *A. elata* plants among sites in different slopes using the methodology by Salifu and Timmer (2003).

Results

The difference of population characteristics

Height of *A. elata* plants was similar in sites of slopes of 5° and 9°, but that on the slope of 14° declined by 51-58% (Table 1). In contrast, soil available P concentration was highest in sites on the slope of 14° (Table 2). Soil pH was higher at the site in slope of 5° than that of 9° by 10%. Otherwise, most parameters about plants and soils in *A. elata* populations were not statistically different among sites of the three slopes.

Principle component analysis

The first PC accounted for 31.14% of the data variation, while the second PC accounted for the proportion of 21.75%. Therefore, the first two PCs together accounted for over 50% of cumulative variation of data. For *A. elata* plants, the first PC can be described as adverse properties of height growth in high density and diameter growth with dense leaf growth in a given area, while the second PC can be taken as the adverse relationship between diameter and density (Fig. 1). In the first axis, *A. elata* height, leaf weight and plant density were loaded with higher values of total N and P concentrations in soils, enzyme activities for N and P assimilation, and leaf N concentration. In contrast, RCD

and leaf cover ratio were accompanied by higher loadings of P availability (leaf P and soil available P concentration) but by negative loadings of leaf N concentration. In the second axis, the density was accompanied by higher values of soil nitrate-N concentration but lower values of leaf sugar and soil pH value. These two parameter, however, were positively loaded with RCD.

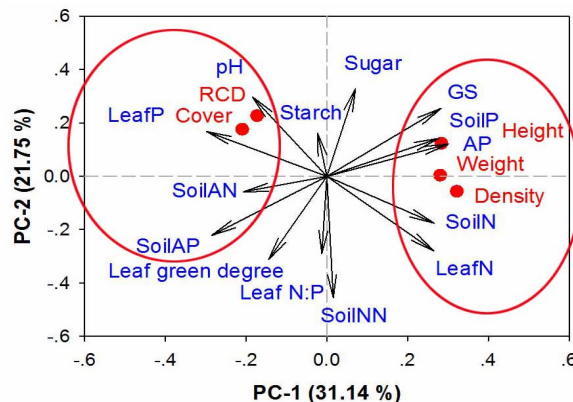


Fig. 1. The principle component analysis on density, growth, leaf parameters and soil properties in sites of *Aralia elata* (Miq.) Seem. plants. Red dots indicate growth and density in *A. elata*. RCD, root-collar diameter; cover, leaf cover ratio in a given area (1m × 1m). Characters in blue colour at the end of arrows indicate leaf and soils parameters. GS, the glutamine synthase activity; AP, acid phosphatase activity; Leaf N, N concentration in leaves; Leaf P, P concentration in leaves; Sugar, soluble sugar concentration in leaves; Starch, starch concentration in leaves; Leaf N:P, foliar N:P ratio; pH, soil pH value; SoilAN, ammonium-N concentration in soils; SoilINN, nitrate-N concentration soils

Table 1. Density, growth, and leaf parameters of *Aralia elata* among sites on slopes in degrees of 5°, 9° and 14°

| Parameters | Slope | | | ANOVA | |
|-----------------------------------------------------------------------|---------------|--------------|--------------|---------|--------|
| | 5° | 9° | 14° | F value | Pr>F |
| Density (clumps m ⁻²) | 0.36±0.13 | 0.78±0.23 | 0.42±0.30 | 2.59 | 0.1542 |
| Cover ratio (percent m ⁻²) | 60.11±8.47 | 43.61±5.92 | 65.56±13.60 | 3.61 | 0.0935 |
| Height (m) | 1.63±0.17a | 1.88±0.33a | 0.80±0.38b | 9.25 | 0.0147 |
| RCD (cm) | 3.33±0.55 | 2.33±0.55 | 3.00±0.95 | 1.40 | 0.3170 |
| Leaf weight (g m ⁻²) | 14.31±7.67 | 21.52±9.56 | 13.16±1.15 | 1.09 | 0.3943 |
| Leaf N (mg g ⁻¹) | 15.91±1.65 | 23.02±2.24 | 19.49±4.07 | 4.20 | 0.0723 |
| Leaf P (mg g ⁻¹) | 3.14±0.74 | 1.95±0.09 | 2.71±0.78 | 2.52 | 0.1609 |
| Leaf N:P ratio | 6.43±1.18 | 12.77±2.66 | 9.93±3.47 | 3.96 | 0.0801 |
| Leaf GS (A _{50nm} mg ⁻¹ protein h ⁻¹) | 0.15±0.04 | 0.16±0.08 | 0.07±0.02 | 2.72 | 0.1442 |
| Leaf ACP (µg _{NPP} g ⁻¹ FW min ⁻¹) | 15.82±4.34 | 27.77±12.71 | 16.05±1.93 | 2.05 | 0.2098 |
| Leaf sugars (mg g ⁻¹) | 432.83±178.29 | 411.39±79.18 | 357.17±81.25 | 0.27 | 0.7689 |
| Leaf starch (mg g ⁻¹) | 63.44±18.10 | 66.11±15.93 | 74.67±8.62 | 0.42 | 0.6734 |

Table 2. Soil parameters of *Aralia elata* populations among sites of slopes in degrees of 5°, 9° and 14°

| Soil parameters | Slope | | | ANOVA | |
|------------------------------------|------------|------------|-------------|---------|--------|
| | 5° | 9° | 14° | F value | Pr>F |
| Total N (mg g ⁻¹) | 1.69±0.80 | 2.95±0.6 | 2.04±0.50 | 2.20 | 0.1830 |
| Total P (mg g ⁻¹) | 5.06±0.77 | 5.31±0.39 | 4.59±0.20 | 1.36 | 0.3260 |
| Ammonium N (mg kg ⁻¹) | 0.21±0.18 | 0.01±0.01 | 0.49±0.39 | 2.52 | 0.1605 |
| Nitrate N (mg kg ⁻¹) | 2.97±1.05 | 4.75±1.40 | 4.37±0.44 | 2.17 | 0.1950 |
| Available P (mg kg ⁻¹) | 5.53±0.80b | 4.07±4.42b | 16.19±4.20a | 9.32 | 0.0144 |
| pH | 5.90±0.30a | 5.34±0.06b | 5.64±0.13ab | 5.54 | 0.0434 |

Correlation analysis

The Pearson correlation indicated significant relationship between density of the *A. elata* population and both leaf N ($n = 9$; $R = 0.6953$; $P = 0.0376$) and P concentrations ($n = 9$; $R = -0.7111$; $P = 0.0317$). Therefore, the fitted lines in the scattered plots revealed a positive linear regression between leaf N and the density (Fig. 2A) and a negative linear regression between leaf P concentration and the density (Fig. 2B). However, the Spearman correlation was found between leaf ACP and leaf weight ($n = 9$; $R = 0.7936$; $P = 0.0107$). Furthermore, a specific exponential growth model was found for the scattered plots between leaf ACP and weight (Fig. 2C). The relationship between soil pH value and the leaf cover ratio was also indicated to be significant by a Pearson correlation ($n = 9$; $R = 0.7180$; $P = 0.0294$) and a linear regression was found for the scattered plots (Fig. 2D).

The change of leaf N and P concentrations among sites in different slopes

Although the leaf N:P ratio was not statistically different among the sites in different slopes (Table 1), the values tended to increase from the 5°-slope site to the 9°-slope site and showed a declined tendency thereafter. Specifically, the increasing tendency of leaf N:P ratio from the 5°-slope to the 9°-slope resulted from the increase in leaf N concentration and the decrease in leaf P concentration (Fig. 3). Subsequently, the decreasing tendency of leaf N:P ratio from the 9°-slope to the 14°-slope resulted from the decrease in leaf N concentration but the increase in leaf P concentration (Fig. 3). As a result, compared to leaf N:P

ratio at the 5°-slope sites, that at the 14°-slope sites tended to be higher due to the tendencies of increased N concentration and decreased P concentration.

The N and P limitations identified by the vector analysis

Relative to the *A. elata* plants in the 5°-slope, those in the 9°-slope tended to have higher N content and concentration in leaves and greater leaf biomass. Therefore, *A. elata* plants in the 5°-slope showed relative N limit by deficiency to those in the 9°-slope (Fig. 4A). Although *A. elata* plants in the 14°-slope tended to have higher N content and concentration in leaves relative to those in the 5°-slope, they have less leaf biomass. Hence, *A. elata* plants in the 14°-slope were diagnosed to have absorbed excessive N relative to those in the 5°-slope (Fig. 4A). Similarly, *A. elata* plants in the 14°-slope were diagnosed to have the antagonistic symptom relative to those in the 9°-slope because of the decline in N content, N concentration, and leaf weight (Fig. 4A).

A. elata plants in the 9°-slope tended to have lower P content and concentration in leaves but greater leaf biomass relative to those the 5°-slope. Therefore, plants in the 9°-slope showed the relatively symptom of P depletion (Fig. 4B). Relative to *A. elata* plants in the 9°-slope, those in the 14°-slope tended to have lower N content and less biomass but higher N concentration in leaves. This was also characterized as the relative symptom of P toxicity in plants in the 14°-slope (Fig. 4B). Similarly, *A. elata* plants in the 14°-slope were diagnosed to have the relative symptom of antagonistic P uptake to those in the 5°-slope.

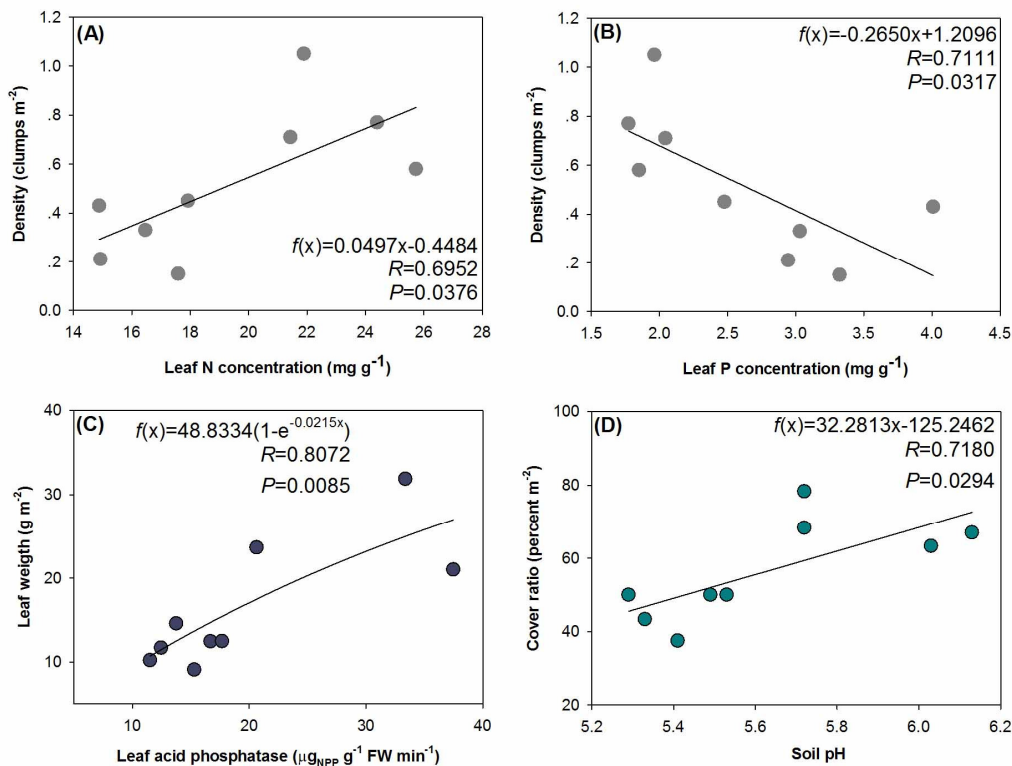


Fig. 2. The correlations between density of leaf N (A) and P (B) concentrations, leaf acid phosphatase and leaf weight (C), and soil pH and leaf cover ratio (D)

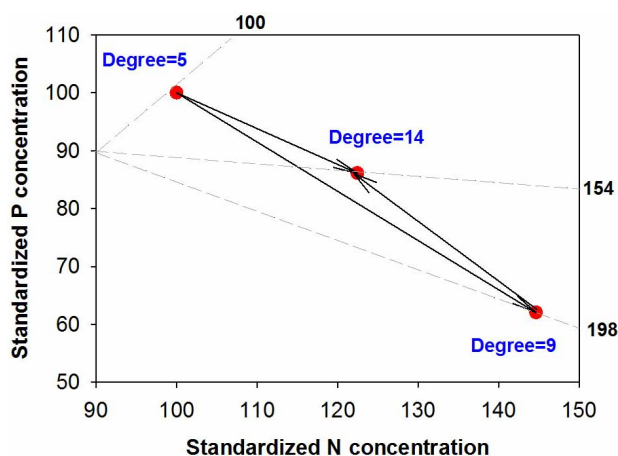


Fig. 3. The contribution of N concentration (x axis) and P concentration (y axis) to the N:P ratio in leaves of *Aralia elata* (Miq.) Seem. plants distributed on slopes of 5°, 9°, and 14° degrees. Arrows indicate the relative changes of N:P ratios from the 5°- or 9°-degree slopes to the 9°- or 14°-degree slopes, respectively. Numbers beyond the end of the dashed lines indicate relative standardized biomass with the one on the 5° slope as the reference (the value of 100)

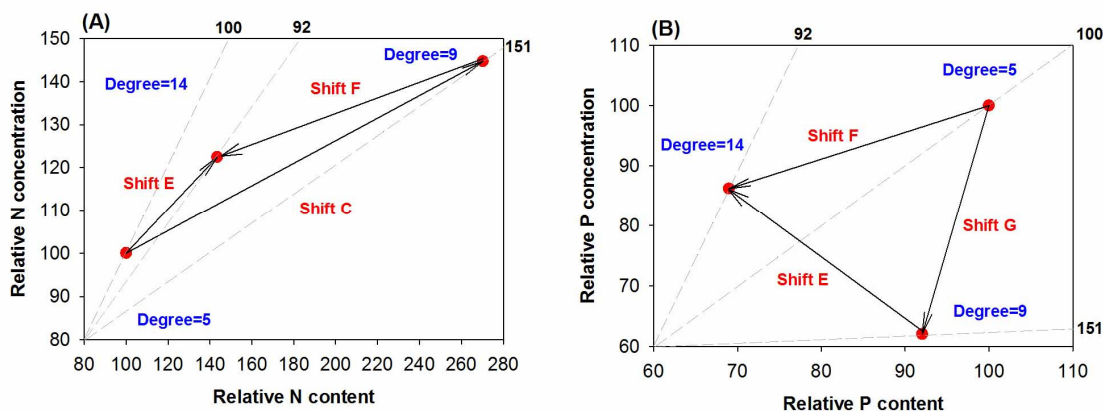


Fig. 4. Vector analysis on N (A) and P (B) in leaves of *Aralia elata* (Miq.) Seem. plants distributed on slopes of 5°, 9°, and 14° degrees. Arrows indicate the relative nutritional states from the 5°- or 9°-degree slopes to the 9°- or 14°-degree slopes, respectively. Numbers beyond the end of the dashed lines indicate relative standardized biomass with the one on the 5° slope as the reference (the value of 100). Interpretations for the vector shifts are adapted from Salifu and Timmer (2003). Shift C, deficiency; Shift E and F, excess; Shift G, depletion

Discussion

Individual growth

In our study, *A. elata* height decreased at the intensity hillslope degree of 14° relative to that on the slope of 5° and 9°. As an early-successional species, *Aralia* spp. plants can exhibit rapid stem elongation to quickly erect a crown and shade out the competitors of other woody plants (Ducey *et al.*, 1996). Pioneer species may be more abundant in steeper than lower slope sites because sloppy terrains have more frequent and larger gaps resulting from landslips (Bianchini *et al.*, 2010). Some *Aralia* spp. plants were found to have a strong ability to elongate trunk with a branched crown on sleepy hillslopes (Briand *et al.*, 1999). Several environmental factors may account for the height decline of *A. elata* on steeper slopes. Firstly, the sites on the steep slope had the shallow soil layer which tended to limit the growth of *A. elata*. Wang (2012) reported that height growth of *A.*

continentalis Kitagawa increased significantly with the increase of soil thickness. Secondly, *A. elata* may prefer the moist, open forest sites than dry, dark forest sites (Whitman *et al.*, 1998). The lower-degree slopes tended to have greater soil moisture but the higher-degree slopes were more prone to have drought condition (Takahashi *et al.*, 2010). Despite the insignificance of our data, leaf GS tended to be lowest in sites on the slope of 14° degree (Table 1) which is generally one of the remarkable responses caused by water deficiency for N assimilation in juvenile woody plants (Mena-Petite *et al.*, 2006). Our data also indicated the tendencies of higher starch concentration but lower sugar concentration in sites on the steep slope. These were coincided with the drought-induced responses in white lupin (*Lupinus albus*) and common reed (*Phragmites australis*) (Thalmann and Santelia, 2017). Finally, the slope controls the velocity of wind (Bai *et al.*, 2004). The higher wind velocity on the steep slope would also limit the height growth of *A. elata*.

The population density

We did not observe the significant difference of *A. elata* density among sites of different slope degrees. Similarly, Li et al. (2013) also reported that the slope degree had no effect on density of arrow bamboo (*Fargesia nitida* and *F. denudate*) seedlings in the understory community. Historical records about *Aralia* plant community showed that the density could be significantly decreased by forest fire (Ducey et al., 1996) or varied between different regenerating forms of seedlings and sprouts (Goto et al., 1996). These results together suggest that the difference of slope degree appear not to be an intensive ecological disturbance to change the density of *A. elata* plants. However, the contributions to the data variation by density and height were of the similar tendency (Fig. 1), which was the result of inherent allocation for light source. The inverse data distribution of diameter growth was the result of trade-off between height and diameter growth in a high density community.

N and P availabilities

Thus foliar N and P concentrations had inverse relationships with density (Fig. 2A, B). To our knowledge, very little evidence can be found about foliar nutrient concentration and density for understory NWFP species. However, at least three possible explanations can account for our results about foliar nutrient and density in *A. elata* plants. Firstly, N deposition increased N availability, stimulating primary production and therefore the biotic P demand (Weand et al., 2010; von Oheimb et al., 2010). This can also be supported by the opposite data distributions between leaf N and P concentrations but similar soil total N and P contents (Fig. 1). Wang et al. (1996) fertilized understory plants in a 10-year-old *Eucalyptus regnans* forest and found that foliage and stem P concentration was lowest with solely N addition. Secondly, deposition-related acidity may have mobilized soil aluminium and iron and therefore reduced the P availability (Weand et al., 2010). This was partly supported by our results that lower soil pH was correlated with lower leaf cover ratio reflecting the decline of leaf growth (Fig. 2D) which can be accompanied by the decrease of P assimilation by of leaves (Fig. 2C). Thirdly, leaf N:P ratio was positively correlated with the water-use efficiency (Cernusak et al., 2010) which varied as a function of N availability but not as a function of P availability (Garrish et al., 2010). In the preceding context, the lower height of *A. elata* plants on the slope of 14° than those on the 5° and 9° degrees may be related to the lower soil moisture on steeper hillslope. Therefore, both correlations between foliar nutrient (N/P) concentration and density may be the results along the soil moisture gradient. Future works are suggested to be conducted under the controlled environment to verify the specific mechanism of our results.

The critical foliar N:P ratio

The foliar N:P ratio in our study ranged between 6 and 13. According to the critical N:P ratios by Koerselman and Meuleman (1996), the *A. elata* communities in our study were all limited by N availability because the N:P ratio was lower than 14. However, Güsewell (2004) summarized that

in various types of vegetation, the maximal species density was found at intermediate N:P ratios between 6 and 20. Current understanding about the critical N:P ratio were mainly originated from studies with N and P addition to the ecosystem in a given region (Tessier and Raynal, 2003; Drenovsky and Richards, 2004; Weand et al., 2010; Mo et al., 2015; Mao et al., 2016; Jing et al., 2017). In these studies, the limitation by N or P was indicated when biomass production was found to increase in response to N or P additions. Therefore, these conclusions have to be given with the necessary pre-condition that the tested ecosystem fell in the status of nutrient deficiency by either N or P. However, this kind of identification for N or P limitation may be bias by the estimate of initial nutrient availability of sites. Actually, it is very hard to choose a highly controlled ecosystem with specific nutrient limitation. Further, according to the classic model of nutrient demand by plants, the deficiency stage with biomass accumulation in response to nutrient addition is only one type of nutritional stages, some other stages, such as luxury consumption and saturation, have also been mentioned on the community level (Koerselman and Meuleman, 1996; Jing et al., 2017). However, to our knowledge only one study has estimated nutrient states at all possible stages using the method of vector analysis at the site level (Oheimb et al., 2010).

Foliar N and P stoichiometry

In more details about our data, the N:P ratio tended to increase from 6 to 13 in the site on 9°-degree slope compared to that on 5°-degree slope, respectively (Table 1). This relative change resulted from meanwhile the tendencies of both N concentration increase and P concentration with leaf biomass increase (Fig. 3). This change can also be described by the meanwhile N deficiency and P depletion (Fig. 4). Here the P depletion was mainly formed by the stimulation of leaf biomass which was driven by N change. Furthermore, the N:P ratio tended to decline from 13 to 10 in the site on 14°-degree slope compared to that on 9°-degree slope (Table 1). This change indicates the relative excess of both N and P mainly due to the decrease of leaf biomass (Fig. 4) although the P concentration increased (Fig. 3). Similarly, both N and P were also characterized to be excess in the site on 14°-degree slope compared to that on 5°-degree slope due to the decrease of leaf biomass (Fig. 4). Therefore, the N and P states on the community level were more complicated than the critical N:P ratio at least in our case.

Conclusions

Among *A. elata* communities on different slope degrees, those on the slope in 9°-degree had highest individual height in soils with low pH value and P availability. N availability tended to promote population density and individual height growth, while P availability tended to depress density but promote individual diameter growth. Foliar N:P ratio of *A. elata* on 9°-degree slope was nearly 13:1, which was generated by both deficiency-driving N uptake and excessive P depletion. The 14°-degree slope was unsuitable for the distribution of *A. elata* plants and the 9°-degree slope was recommended to develop the natural *A. elata* community as the NWFP source. Thus, some more N might be added to

natural *A. elata* communities at the 9°-degree slope if population density and individual growth were achieved.

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Conflicts of interest

The authors declare that there are no conflicts of interest related to this article.

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