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Nutrient limitation and stable nitrogen isotope ratios in two pioneer species *Robinia pseudoacacia* and *Salix gilgiana* colonized on the nutrient poor sediment bar of a regulated river

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Abstract: Flow regulation through dam construction in the upstream of large rivers enables the colonization of vegetation on the downstream sediment bar with organic matter accumulation and silt trap. *Robinia pseudoacacia*, a woody legume and *Salix gilgiania*, a flood-tolerant shrub colonize on the mid-stream sediment bar where soil nutrient levels are relatively low. This two species can colonize along the gradients of the sediment bar with distinct soil characteristics. Total nitrogen (TN) to total phosphorus (TP) ratios has been widely used as diagnostic indicators of nitrogen saturation and limitation of plant growth. Samples of above-ground organs, roots and soils were collected and analyzed. High TN content was found in nodule followed by leaf, root and then shoot tissue of *R. pseudoacacia* whereas in *S. gilgiania,* it was the highest in leaf followed by shoot and the root tissue. TP content was found high in the leaf tissues of both species. High ratio of nitrogen and phosphorus in the tissue of *R. pseudoacacia* indicating that plant growth is P limited whereas the ratio was very low in the soil samples. The growth of *S. gilgiania* is nitrogen limited as the N and P ratio in the plant tissues was low. Mean nitrogen stable isotope ratio $\delta^{15}N$ in *S. gilgiania* was relatively higher than the *R. pseudoacacia*. Nitrogen saturation in the tissues of *R. pseudo-acacia* occurs as the symbiotic bacteria fix atmospheric nitrogen.

Keywords: *Robinia pseudoacacia*, *Salix gilgiania*, sediment bar, N:P ratio, $\delta^{15}N$.

Introduction

It is widely recognized that large dams can result in a significantly altered shoreline and riparian vegetation both in the impounded area (Springuel et al. 1991) and in downstream reaches (Merritt and Cooper 2000). Depending on the climatic, geological and hydrological characteristics of the local area and the intensity of alteration from the original condition, dam construction can either increase or reduce downstream vegetation (Asaeda et al. 2008). Azami et al. (2004) observed that downstream river channels became forested subsequent to dam construction. In regulated rivers, sediment bars are gradually stabilized by herbaceous vegetation then progressively succeed into more stable phases such as forests (Decamps and Tabacchi 1994).

Salix spp. are among the dominant pioneer tree species in riparian habitats throughout the temperate and subarctic zones in the Northern Hemisphere (Ishikawa 1988; Cooper and van Haveren 1994). *Salix gilgiana* is one of the dominant plants in the sediment bar vegetation of the middle reaches of rivers in central Japan. This species can easily colonizes in sandy habitats, where soil nutrient levels are low and it shows high potential for production. *Robinia pseudoacacia* L., known as black locust in the world is a legume tree native of North America, generally adapted to temperate regions (Hanover and Mebrahtu 1996). It is currently used for production of timber, fodder and honey, and also in pulp industry (Hanover and Mebrahtu 1996; Keresztesi 1980). In addition, it can colonise disturbed sites due to its fast growth and its capacity to fix atmospheric nitrogen by symbiotic association with *Rhizobium* spp.

Nutrient concentrations in plant biomass have been widely used to assess the availability of nutrients to plants and the degree to which particular nutrients are limiting for plant growth (Güsewell and Koerselman 2002). The dependence of nutrient concentrations on nutrient availability may differ between species from nutrient-poor and nutrient-rich sites (Chapin 1980; Garnier 1998). Such interspecific variations make the use of N and P concentrations as indicators of nutrient availability. Ratios of nitrogen to phosphorus (N:P) in plant foliage have been used to assess nutrient limitation in wetland ecosystems and to indicate nitrogen saturation (Tessier and Raynal 2003). The use of stable isotope techniques in plant ecological research has grown steadily during the past two decades. The natural ^{15}N abundance of N_2-fixing plants has been reported to be generally lower than that of nonfixing plants and rather close to the ^{15}N abundance of atmospheric N_2 (Delwiche et al. 1979; Kohl et al. 1979). This paper investigates the levels of nutrients (N and P) and their ratios in the plant tissues to identify the vegetative growth limitation in different colonizing locations along the gradient of a sediment bar. It also aims at understanding the levels of ^{15}N natural abundance in plant tissues and rhizosphere soil to identify the sources of N in the nutrient poor bar.

Study area

The studied sediment bar (Figure 1) is in the middle reach of Arakawa River at Kumagaya city, Saitama Prefecture, Japan (36°08′16" N; 139°20′32" E). The Arakawa River that originates at an elevation of 2475 m a.s.l. on Mt. Kobushigatake, flows 173 km to the sea (i.e. Tokyo Bay) through Chichibu Basin and the Kanto Plain. The sediment bar is located about 80 km from the river mouth at Tokyo Bay and about 60 km downstream from three dams of the mainstream and tributaries, which have operated since 1961 (Futuse Dam), 1999 (Urayama Dam) and 2006 (Takizawa Dam; start of impoundment), respectively. The river slope at the study area was about 1/360, estimated based on the elevation of the riverbeds at 1 km upstream and downstream of the bar. The climate of the study area is typical of

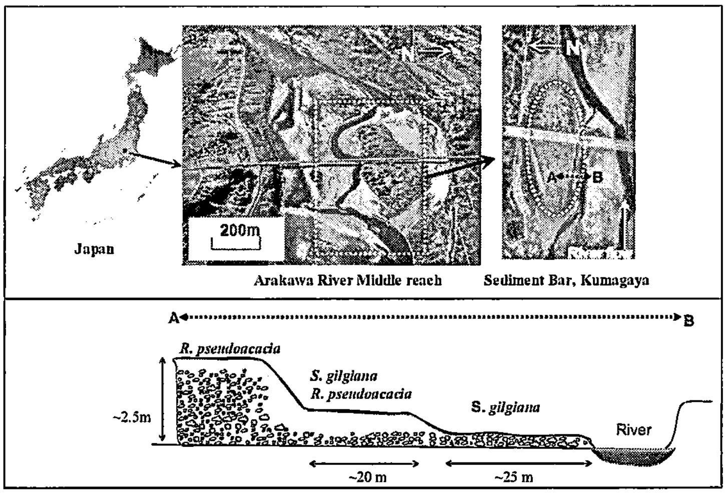

Figure 1. The study area showing the location of sediment bar (a) and sampling points along the gradient of the bar (b).

the Asian Monsoon zone, it is characterized by warm, humid summers and cold dry winters accompanied by strong seasonal winds (Asaeda et al.,2008). The sediment bar was about 700 m long and 250 m wide and the height of the bar was around 2.5 m at maximum from the normal water level of the river channel. There exists another bar on the southern side of the studied bar and the river flows along that bar during normal water flow. When the water level increases the flow along the northern side of the bar becomes active. There are three distinct belts along the slope of the bar from the river channel to the highest point. The most river side belt is about 25 m wide and is dominated by *Salix* spp. such as *S. gilginana*. The middle part (20 m wide) is dominated by both *Salix* spp. and *R. pseudoacacia*. The highest part of the bar is relatively composed of finer sediment and dominated by *R. psudoacacia*, *Albizia julibrissin*, *Juglans ailanthifolia* along with other herbaceous and grass communities.

Methods

In April, 2009 same aged trees from each belt were selected and marked. Samples of above ground parts, below ground parts of the two species were collected every month from April to June 2009. We collected three plant samples of each species from each zone and the shoots were divided into 50 cm segments (the data presented here of shoots and leaves were taken from 1 m height). Along with the root samples of *R. pseudoacacia*, nodules were also collected. All of these samples were stored in plastic bags for transportation to the laboratory. At the same time, soil samples (1–20 cm depth) were collected and were tightly sealed in a plastic bag.

In the laboratory, roots and nodule samples were rinsed with water and were dried along with other plant samples at 80°C in the oven for more than 3 days until the weight was constant. Oven-dried samples were ground with a Wiley mill and were stored in sealed plastic vials until chemical analyses were conducted. Soil samples were air-dried, then the particle sizes were determined using sieves, according to the ASTM D422-63, 2002.

For the plant biomass, nitrogen (N) was determined with a Yanoco MT5 CHN analyzer (Kyoto, Japan). Phosphorus (P) content was determined by the molybdenum blue colorimetric method (Murphy and Riley 1962) subsequent to digestion with potassium persulfate (APHA, 1998) in an autoclave (120°C for 3 hrs). For soil samples (<0.5 mm), N and P was determined by the same method as used for plant biomass. Total inorganic P was determined by the ignition method (Kuo, 1996). Samples of ignited (550°C, 1 h) soil were extracted for 16 h with 0.5 M H_2SO_4. The TN and TP of soil obtained for each colonizing location were multiplied by the percentage of particles finer than 0.5 mm, and were then referred to as modified TN and TP (MTP, MTN). Moisture content was determined as weight loss after drying at 105°C for 24 h.

To determine the ratios of stable nitrogen isotopes, samples were combusted at 950°C in an elemental analyzer (Elementar Analysensysteme Gmbh), and the combustion products (N_2) were introduced to an isotope-ratio mass spectrometer (Isoprime) with a He carrier. The ^{15}N: ^{14}N ratio

($\delta^{15}N$) was expressed relative to N_2 in air. It was calculated as:

$$\delta^{15}N = \left\{ \frac{R(\text{sanpule})}{R(\text{stndard})} - 1 \right\} \times 1000\ (‰)$$

where $R = {}^{15}N/{}^{14}N$.

Results and discussion

Soil characteristics and nutrient condition

Table 1. Quartiles, sorting coefficient (So) and Skewness (Sk) of sediment particle sizes (mm) at tree colonizing locations along the gradients of the sediment bar. Standard deviations are observed to less than 20% (data not shown)

	d_{25}	d_{50}	d_{75}	So	Sk
R. pseudoacacia upper	0.122	0.217	0.429	1.87	1.12
R. pseudoacacia down	0.262	0.493	1.356	2.27	1.46
S. gilgiana upper	0.632	1.001	1.351	1.46	0.85
S. gilgiana down	2.887	7.821	23.865	2.87	1.13

The quartiles, sorting coefficient (So) and skewness (Sk) of sediment particle sizes (mm) at colonizing locations of each studied species are shown in Table 1. The median particle size (d_{50}) in *Salix* down part was greater than 7 mm where as in *Robinia* upper part it is less than 0.25 mm which implies distinct substrate characteristics among the sites studied. Thus the sediment particle size of *R. pseudoacacia* sites was significantly smaller than those of *S. gilgiana* sites (t-test, $p<0.05$). The surface layer was coarser in the order *Salix* down, *Salix* upper, *Robinia* down and *Robinia* upper. Average water contents of the rhizosphere soil of the study sites are shown in Figure 2. Water content in normal weather conditions was higher at fine sediment sites than at coarse sediment sites, because fine sediment has relatively lower permeability and evaporation rates.

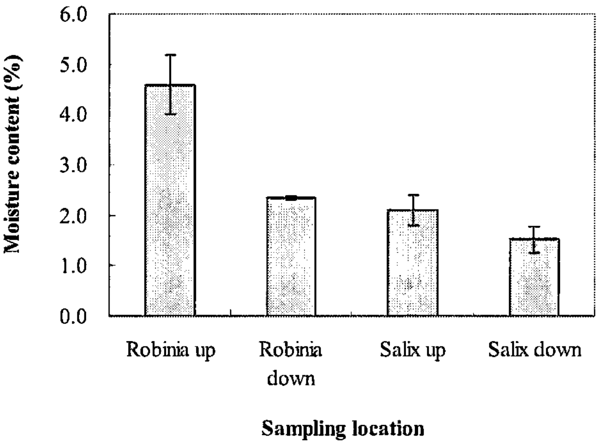

Figure 2. Average moisture content of sediment at different sampling locations. Error bars represent one SE above and below the mean.

The nutrient content in soil is low and the modified values decreased downward with increasing particle sizes (Table 2). Total nitrogen (TN) content varied from 250–805 mg/kg at *Robinia* sites while it was very low at *Salix* sites. Soil nutrient contents decreased from April to June which was attributed to the uptake of nutrients by plants during growing season. Relatively lower ratios of N and P were recorded in soil samples (0.75–3.24).

Table 2. Modified[1] values for total nitrogen (TN), total phosphorus (TP) and inorganic phosphorus (IP) of different colonizing location of RP (*R. pseudoacacia*) and SG (*Salix gilgiana*). [1]Values are obtained by computing the product of nutrient content in particles less than 0.5 mm (mg/kg) and the fraction of particles (%) less than 0.5 mm in the rhizosphere. The particle fraction (%) less than 0.5 mm in the four locations are: 80.55 (RP upper), 50.97 (RP down), 19.49 (SG upper) and 7.10% (SG down). Standard deviations are indicated after the mean valu

	TN			TP			IP	
	April	May	June	April	May	June	April	June
RP upper	805.6±80.5	590.8±87.9	483.4±79.3	464.6±67.1	392.8±63.7	385.0±32.6	191.0±21.8	165.1±32.6
RP down	356.8±50.9	271.8±29.4	254.9±50.9	270.48±30.7	206.8±31.3	283.5±46.2	119.41±15.6	80.02±12.6
SG upper	214.5±39.0	136.5±39.1	71.5±11.0	92.59±3.54	76.04±5.36	82.02±9.18	47.7±4.6	28.4±3.8
SG down	80.4±22.8	31.9±3.5	30.7±4.0	52.68±7.88	42.63±13.3	27.79±1.69	11.1±3.4	11.4±2.9

Changes in nutrient concentrations in plant organs

Figure 3 and 4 show changes in N and P concentrations in each plant organ of *R. pseudoacacia* and *S. gilgiana* from April to June. The same pattern was also found in the organs of the plants colonizing on the lower slope of the bar (data not shown). Concentrations in leaves decreased rapidly from April to May, the leaf expansion period. The results show that the largest proportion of P was distributed in leaves of both species throughout the study period. The highest concentration of N was found in the nodule of *R. pseudoacacia* while in *S. gilgiana* it was highest in the leaves. The pattern of change in leaf nutrient concentration is similar to that of other deciduous trees reported previously (e.g., Grigal et al. 1976; Chapin et al. 1980; Sakio and Masuzawa 1992). The initial decline in

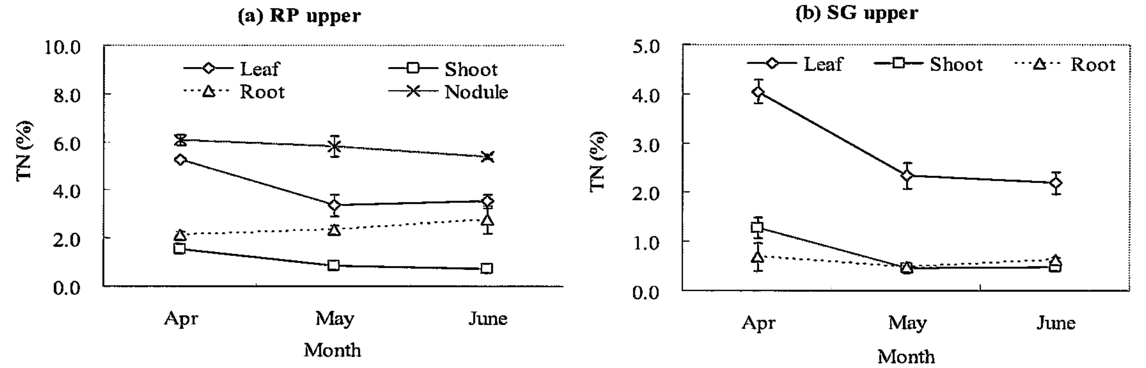

Figure 3. Total nitrogen content in the organs of (a) *R. pseudoacacia* (RP) and (b) *S. gilgiana* (SG). Error bars represent one SE above and below the mean.

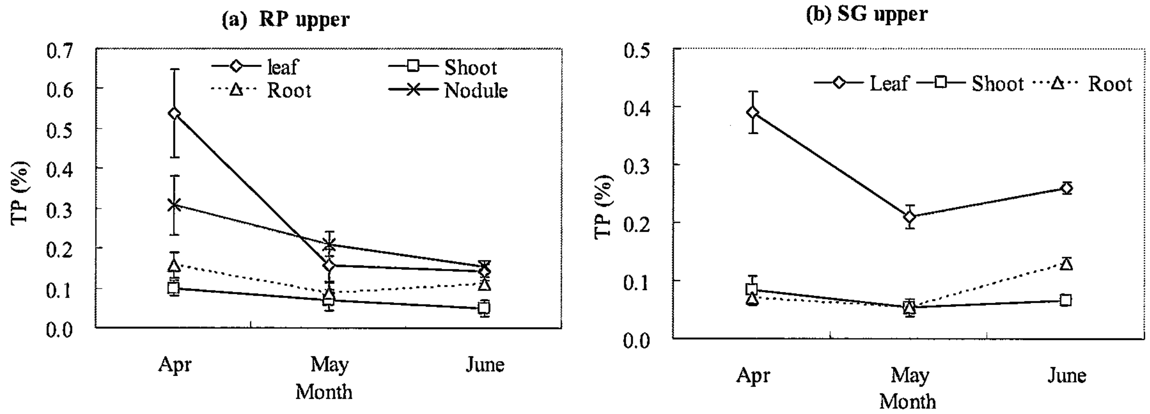

Figure 4. Total phosphorus content in the organs of (a) *R. pseudoacacia* (RP) and (b) *S. gilgiana* (SG). Error bars represent one SE above and below the mean.

concentrations of N and P in leaves coincided with leaf expansion and was probably due to dilution by increasing leaf material (Grigal et al. 1976) and leaching loss by rain might also contribute to the decline.

Foliar N:P ratios and nutrient limitation

Nitrogen to phosphorus (N:P) ratios have been widely used as diagnostic indicators of nitrogen saturation (Fenn et al. 1996) and limitation of vegetative growth by these nutrients (Penning de Vries et al. 1980). More recently, N:P ratios have been applied to identify thresholds of nutrient limitation. Based on a review of 40 fertilization studies, Koerselman and Meuleman (1996) showed that the tissue N/P ratio >16 indicates P limitation, while N/P ratio <14 is indicative of N limitation. Our study shows that the N/P ratio in leaves of *S. gilgiana* was <14 which indicates N limited growth (Figure 5). The ratio of N/P in leaves of *R. pseudoacacia* was found higher than 16 (except in April when the P concentration was high in new leaves) indicate N saturation and P limitated growth.

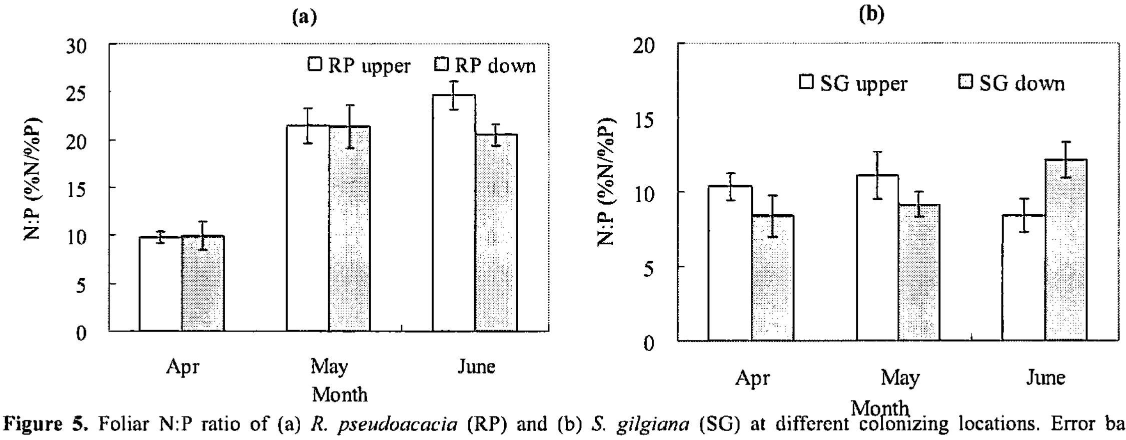

Figure 5. Foliar N:P ratio of (a) *R. pseudoacacia* (RP) and (b) *S. gilgiana* (SG) at different colonizing locations. Error bars represent one SE above and below the mean.

Levels of $\delta^{15}N$ in plant organs and soil

In our study, the levels of $\delta^{15}N$ in the organs were found higher in *S. gilgiana* than N_2-fixing woody legume, *R. pseudoacacia* (Table 3). The $\delta^{15}N$ values in the organs of *R. pseudoacacia* were close to the atmospheric values. The shoot and root tissues of both species were less enriched in ^{15}N than leaves. Such differences have been attributed to internal recycling of plant N, in that N is first translocated to leaves and then retranslocated to other organs as they grow, and exported N has tendency to become depleted in ^{15}N (Shearer and Kohl 1986). Nodules of most legume species are usually found to be enriched in ^{15}N (Yoneyama et al. 1991) because of the occurrence of isotopic discrimination at the branching point in the utilization of fixed N. The rhizosphere soil of *S. gilgiana* was found to be more enriched in ^{15}N than *R. pseudoacacia.* It relies on the commonly observed phenomenon that soil mineral N is usually slightly naturally enriched in the heavy isotope of N, ^{15}N, compared to atmospheric N_2 (Shearer et al. 1978). The soil from *R. pseudoacacia* area was less enriched in ^{15}N implies the leaching of fixed N from roots and nodules to the surrounding soil.

Table 3. Levels of ^{15}N natural abundance (‰) in plant parts of *R. pseudoacacia* and *S. gilgiana* and in rhizosphere soil. Standard deviations are observed to be <0.6‰.

	$\delta^{15}N$ (‰)	
Plant part	*R. pseudoacacia*	*S. gilgiana*
Leaf	+1.08	+3.37
Shoot	-0.11	+2.63
Root	+0.73	+2.49
Nodule	+1.13	
Soil	+0.53	+3.51

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