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Seasonal dynamics of soil macro- and micronutrients and phenolics under kudzu (*Pueraria lobata*) stands in floodplain of a modified river

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Abstract: Kudzu (*Pueraria lobata*) is a rapidly spreading liana in the floodplain of Tama River, Japan. This species is very rich in secondary metabolites and adds substantial amount of litter into the soil due to its huge biomass turnover in every growing cycle. This study aims to investigate the seasonal dynamics of major macro- and micronutrients and phenolics in soil associated with the litter production and growth of kudzu plants in the downstream of Tama River. Soils were collected from three kudzu-infested spots along the banks of the river and analyzed for total carbon, total nitrogen, total phosphorus, potassium, copper, zinc, sodium and phenolic contents. The patterns of seasonal changes of those soil elements in all three spots were more or less similar. The levels of the nutrients were related with the growth stages of kudzu plants. Concentrations of all nutrients except nitrogen went down when the kudzu was at active vegetative stage. The nutrients were again mineralized into the soil after the decomposition of kudzu litter at winter season. Soil phenolic content under kudzu stand also varied seasonally and the highest quantity was recorded in winter as an outcome of kudzu litter decomposition. Soil phenolics was not found to interfere the chemical properties of soil except K, of which concentration in soil was negatively correlated with that of soil phenolics.

Key words: *Pueraria lobata* ,Litter ,Macro- and micro-nutrients ,Soil phenolics

Introduction

Tama River, originating from the mountains in the western district of Tokyo, flows into Tokyo bay. The river is 138 km long and its catchment basin is 1240 km^2; the average slope is 1/500, which is relatively steep. This river exemplifies problems that are typical of Japanese rivers, which include gravel excavation, dam construction, flow stabilization, and the invasion of alien species. Except for the downstream reach close to the river's mouth, most of the riparian area of the Tama River was originally stony, covered only with ephemeral annuals. However, since the 1960s, the floodplain has gradually received vegetation. Some reasons for this include the construction of a dam and several weirs in the upstream area (Naiman et al. 1998), and a prohibition on the extraction of gravel from the river channel for construction work. Today, one of the most dominant species in the Tama river floodplain is Kudzu (Pueraria montana Lour.).

Kudzu is an invasive legume, has become a great concern in different parts of the world (Sun et al. 2006). This species originated in China, and it is one of the medicinal plants used by traditional Chinese (Keung and Vallee 1998). In 1876, the Japanese government first exhibited kudzu as an ornamental vine (Bhowmik 2005); later on, it was introduced to the United States (Winberry and Jones 1973). By the late 1970s, kudzu was recognized as a common weed in most parts of the world (Everest et al. 1999), and in 1978 it was added to the Federal list of noxious weeds (Boyette et al. 2002). Today, an estimated seven million acres of land are infested with Kudzu in the southeastern United States alone (Stewart 2005).

Kudzu is perennial with large stolon and rhizomes underground (Parks et al. 2002). It is capable of developing multiple canopy layers, with leaf area indices (m^2 leaf area per m^2 ground area) from 3.7 to 7.8 (Wechsler 1977; Tsugawa et al. 1993), equivalent to entire deciduous forest canopies. Leaves die off during the winter and it is still alive and starts growing in the same spot in the spring (Bodner and Hymowitz 2002). This species adds a huge amount (c.a. 900 g DM m^{-2}) of litter to its understory soil (Crespo et al. 2001). Therefore, due to addition of a great deal of organic matter, the kudzu-infested soil should be rich in nutrients. However, there are some notions that usually the soil of kudzu stand is inferior in the physical and chemical properties particularly within the reach of its root, i.e. soil in absorbing root zone (Takahashi et al. 1995). In addition, kudzu contains a substantial amount of phenolic compounds (Kirakosyan et al. 2003) which can be released into the soil environment through litter decomposition (Putnam and Tang 1986). Many researchers have shown that presence of high level of phenolic compounds in soil affects its physical and chemical properties (Batish et al. 2002; Inderjit 2002).

In the light of above facts, we hypothesized that during different growth phases of kudzu, the level of soil nutrients may vary which is important for other neighboring plant species; and soil phenolics associated with kudzu litter decomposition may interfere with the chemical properties of soil; and these may render kudzu competitive ability. Therefore the objectives of the study was to a) to observe the seasonal dynamics of major soil macro- and micronutrients in 3 sites along Tama river with different soil characteristics, and b) to assess whether soil phenolics associated with kudzu litter decomposition affect the nutrient chemistry of soil

Study sites

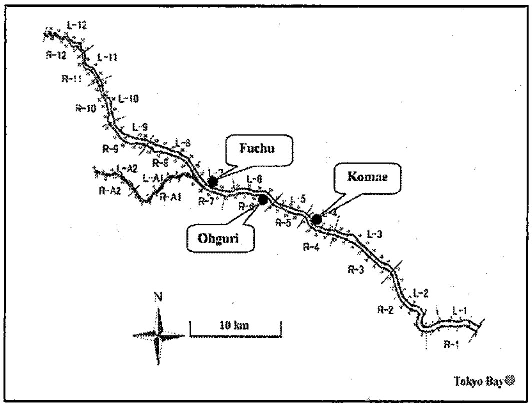

Fig. 1 Location of the study sites downstream of the Tama River, Japan

Observation was conducted at three locations: (1) Fuchu (35°39′46″ N, 139°26′15″ E), (2) Ohguri (35°38″59′ N, 139°28′32″ E), and (3) Komae (35°37′17″ N, 139°34′55″ E) (Fig. 1). These sites are situated at 34.6, 33.6 and 22.1 km up from the river mouth, respectively. All three locations were relatively flat, although slightly inclined from the bank side to the channel. However, these locations are different in height: 1.3–2.3 m at Fuchu, 3.5–4.5 m at Ohguri and 2.6–3.7 m at Komae from the riverbed. Thus, inundation frequency differed among the sites. The Fuchu location was most frequently inundated, followed by Komae, and Ohguri, which was rarely inundated even under high floods. The flood levels and inundation history of these locations are shown in Fig. 2. The largest flood ever experienced in the past 20 years occurred on September 7th,

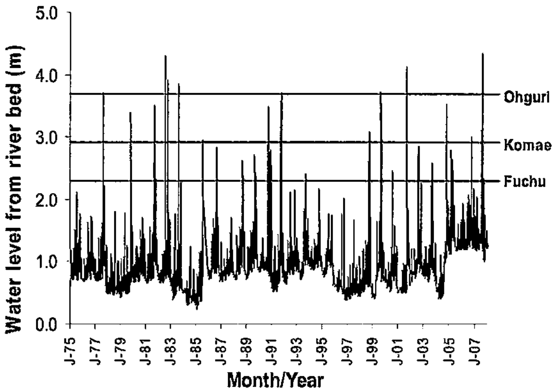

Fig. 2 Recent records of water level height from the Tama riverbed and inundation history of the study sites. Horizontal lines from the top represent the height of Ohguri, Koame and Fuchu, respectively, from the riverbed

2007. During the flood, all of these areas were flooded and large colonies of lianas disappeared. The average temperature at those locations is 15°C, with August being the hottest month and January being the coldest. There is about a 20°C difference between the summer and winter months. There are four distinct seasons: mild spring and fall, hot summer, and winter, with few or no snowfalls. Annual rainfall is around 1500 mm, distributed throughout the year.

The river transports a large amount of sand or other types of finer wash-load, which accumulates on the floodplain during floods and promotes vegetation. Soil properties differ among the different locations depending on the frequency of the inundation. Fuchu is the most sandy among these locations with relatively low plant biomass (61±9 g DW m^{-2}). The higher part of Fuchu is gravelly with overlying thin sand layers. Soil of Komae is also sandy and stony. However, the organic matter content is little higher than Fuchu soil and the depth of soil is higher in the elevated parts. Ohguri soil, on the other hand, is not accumulated by washloads and it was carried away from other places when the adjacent buildings were constructed. Since this place is not experienced frequently by flooding event, the soil is more or less homogenous in physical and chemical natures. The organic matter content is higher than Fuchu and Komae soil and the soil is not stony. The average physicochemical properties of soils of the sites (not infested by kudzu) are presented in Table 1.

Table 1 Physicochemical properties of soils of study sites

	Fuchu	Ohguri	Komae
pH	6.95 ± 0.35	6.29 ± 0.21	6.97 ± 0.24
Organic matter (%)	0.40 ± 0.04	2.21 ± 0.07	0.53 ± 0.03
Total carbon (%)	0.76 ± 0.02	4.3 ± 0.03	1.3 ± 0.01
Total nitrogen (%)	0.30 ± 0.04	0.75 ± 0.05	0.45 ± 0.07
Total phosphorus (%)	0.03 ± 0.003	0.07 ± 0.004	0.03 ± 0.005
Potassium (ppm)*	207.66 ± 15.24	411.28 ± 18.29	235.41 ± 22.64
Magnesium (ppm)*	46.49 ± 9.87	169.01 ± 11.23	59.78 ± 7.19
Zinc (ppm)*	8.50 ± 1.3	18.47 ± 2.9	7.48 ± 1.7
Copper (ppm)*	8.02 ± 0.9	21.94 ± 2.7	9.08 ± 1.3
Sodium (ppm)*	197.29 ± 11.47	255.13 ± 26.54	201.47 ± 18.75
Soil texture	Sandy-loam	Loamy	Sandy-loam

*Extracted by Mehlich 3 extractant

Methods

Soil and plant samples (kudzu) from study sites were collected in every moth starting from April 2008 to August 2009. Samples were collected only in sunny days when there was no precipitation in the sites. For estimation of kudzu biomass, typical ramets and shoots were selected, underground organs and surrounding sediments were carefully dug out to a depth of at least one meter so as to obtain all of the underground tissues. The depth of the Pueraria lobata roots was not significantly different at the same locations. Materials at the bottom of the hole were carefully sieved to ensure there was no remaining plant material. All of these samples were put into a plastic bag for transportation to the laboratory. At the same time, soil samples from the site were collected and air-dried and the particle sizes of the soil samples were determined using sieves according to the American Society for Testing and Materials (ASTM D422-63 2002). Part of each sample was oven dries for nutrient analyses. When the soil samples were not used, they were kept airtight in polyethylene bags.

In the laboratory, all of the plant samples were rinsed with pressurized water. Then they were dried at 80°C in the oven for more than three days until their weight was constant. The total biomass was measured separately for each plant. The total carbon (TC) and total nitrogen (TN) of soil was determined with a Yanoco MT5 CHN analyzer (Kyoto, Japan). The total phosphorus (TP) was determined by the molybdenum blue colorimetric method (Murphy and Riley 1962) after digestion with H_2SO_4–$HClO_4$ (APHA 1998). Potassium, calcium, magnesium, sodium, copper and zinc were extracted by Mehlich-3 extractant (Mehlich 1984) form oven dried soil samples by methods stipulated by Ziadi and Tran (2007). Then they were measured by Atomic Absorption spectrophotometer (AA-6300 Shimadzu, Japan) at respective wavelengths specified for the metals.

For estimation of soil phenolics concentration, soil samples were extracted by adding 250 ml of distilled water and shaking them on a water bath incubator with a shaking action lasting 1 h at room temperature. The extracts were filtered and kept in a freezer until their use. Next, the amount of phenolics in the water was estimated by the Folin-Ciocalteu assay (Singleton and Rossi 1965), with gallic acid as the standard

All statistical analyses were performed using SPSS version 13.0 (SPSS Inc., Chicago, IL). The correlations were evaluated by Pearson's correlation coefficient. All P-values were considered significant at ≤ 0.05.

Results and discussion

The results of this study revealed that the dynamics of major macro- and micronutrients for plants under kudzu stands in Tamagawa floodplain were related to the growth stages of kudzu plant. From the figures, it could be seen that though the level of soil nutrients in three study sites varied during the observation period, their pattern of change over seasons were almost similar. The Fuchu site is the least fertile followed by Komae.

Seasonal yield of kudzu biomass

Kudzu plant start growing on mid-March from its dormant rhizome or rootstock and its growth is very slow (lag phase) until it starts a very rapid vegetative growth on mid-May. Until mid-September, the plant extends its territory with massive vegetative growth and literally covers everything on its way. Then its growth becomes sluggish and maturation of plants starts. At the onset of winter the plants die off and then only its stem over winter in the form of underground stem and rhizome. The seasonal pattern of biomass yield of kudzu is closely related to its growth stage. Figure 3 shows the seasonal biomass turnover of kudzu. The figure depicts that total biomass produced by kudzu at three sites have conspicuous difference. Ohguri yielded the highest biomass of kudzu throughout the observation period. Komae was the lowest in yield, though for a short period in 2008, the biomass value of Komae was recorded

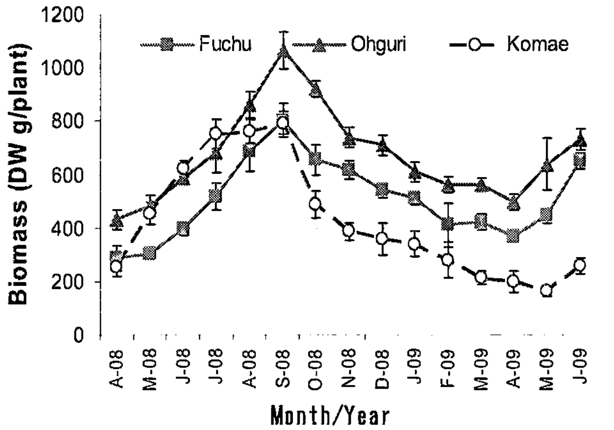

Fig. 3 Seasonal changes of kudzu biomass at study sites. Error bars indicate standard deviation (+1SD)

higher than Fuchu. However, it was distinct that the seasonal pattern of biomass production by kudzu was almost similar in all sites. The highest biomass in all three places was peaked in August.

Seasonal changes of nutrients in soil

Concentrations of major elements (C, N, P, and K) are shown in Fig. 4. We estimated total carbon (TC), total nitrogen (TN) and total phosphorus (TP) in soils for every month from April 2008 to May 2009. However, we could measure the concentrations of potassium and micronutrients in soil only once a season. The sampling moths are May (spring), August (summer), November (autumn) and February (winter). From figure, it can be seen that the concentrations of TC, TN, TP and K in kudzu-infested soils were related to the growth phases of kudzu. The trends of seasonal changes of these major elements in soil were almost similar. From April to August, when kudzu grows vigorously, the concentrations of these elements were very low, though at the later stage of kudzu growth the concentrations were increasing. This might be because kudzu at its maturation stage did not grow further and other species that grew underneath the canopy at its earlier stage were already dead and decomposed. The decomposition has mineralized those elements and thus their concentration in soil was higher than previous months. After November, the plant dies off and incorporated into the soil it

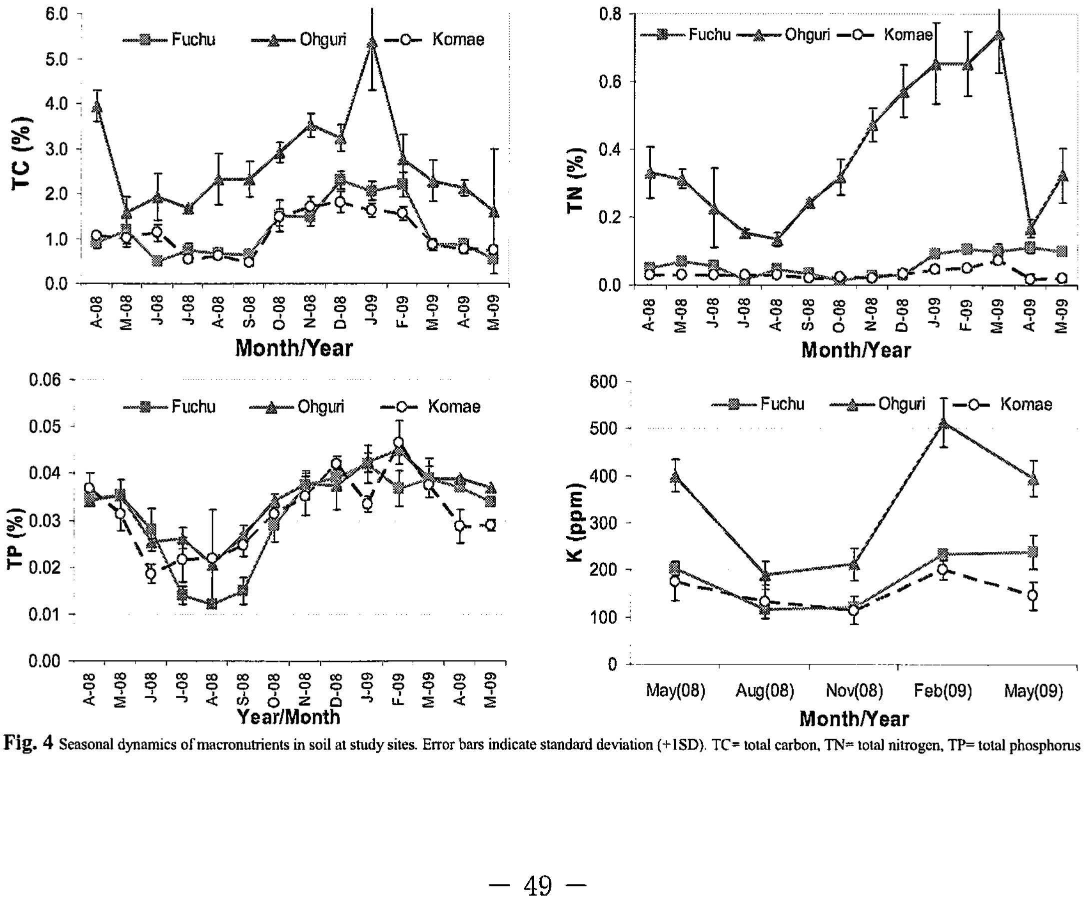

Fig. 4 Seasonal dynamics of macronutrients in soil at study sites. Error bars indicate standard deviation (+1SD). TC= total carbon, TN= total nitrogen, TP= total phosphorus

grow on. Therefore, the highest amounts of C, N, P and K were recorded in winter season. The concentrations of the elements at each sampling time varied due to its inherent soil properties. Ohguri soil always had the highest TC, TN and K. Figure 5 presents the seasonal dynamics of micronutrients (Cu, Zn and Na) in kudzu-infested soil at Fuchu, Komae and Ohguri. The dynamics of trace elements in soil also followed the similar pattern as observed in macronutrients during the observation period. Table 2 shows the correlation between the nutrients elements in soil and kudzu biomass. The correlation analyses show that all soil nutrients except TN had negative correlation with the biomass yield of kudzu. This signifies that when kudzu was at its vigorous growing stage it drained off a huge amount of those nutrients from the soil. In case of nitrogen concentration in soil and kudzu biomass, no significant correlation could be found. This might be due to the symbiotic nature of kudzu. Kudzu is a leguminous plant and it performs symbiosis with Rhizobium bacteria (Edman 1953; Fujita et al. 1993). Therefore, kudzu is able to colonize and proliferate on poor sites where other vegetation is unable to grow (Witkamp et al. 1966; Forseth 2004).

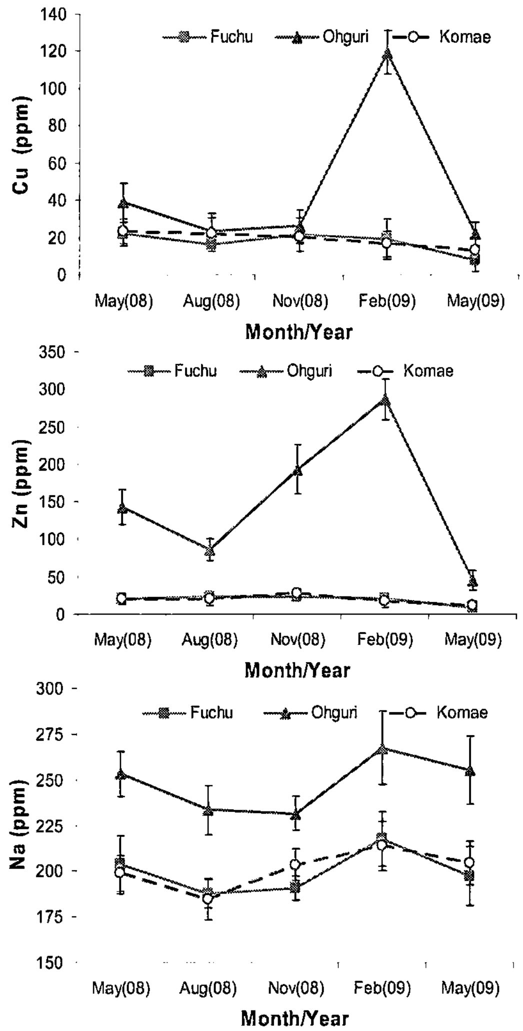

Fig. 5 Seasonal dynamics of micronutrients in soil at study sites. Error bars indicate standard deviation (+1SD)

Table 2 Correlations between nutrient elements and phenolics concentrations in soil and kudzu biomass

Soil nutrients	Soil phenolics	Kudzu biomass
TN	R = +0.41, p = 0.131	R = +0.32, p = 0.254
TC	R = –0.03, p = 0.33	R = –0.48, p = 0.762
TP	R = +0.036, p = 0.899	R = –0.91, p = 0.565
K	R = +0.47, p = 0.076	**R = –0.52, p = 0.047**
Cu	**R = +0.75, p = 0.001**	R = –0.20, p = 0.472
Zn	**R =+ 0.72, p = 0.002**	R = –0.119, p = 0.673
Na	**R = +0.55, p = 0.033**	R = –0.326, p = 0.235
Soil phenolics	—	R = +0.172, p = 0.539

+, positive correlation; –, negative correlation, values with bolt letters are statistically significant at P<0.05

Phenolics in soil

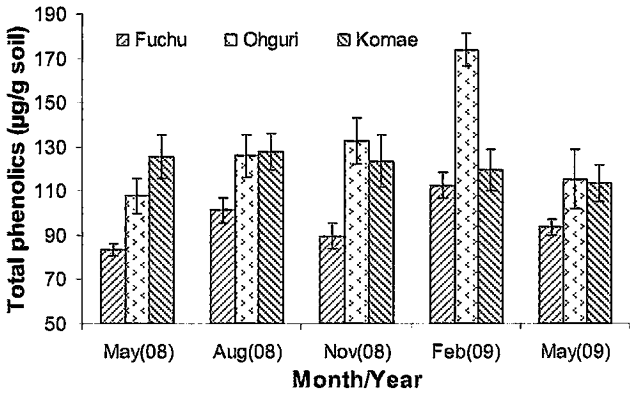

Fig. 6 Seasonal dynamics of soil phenolics at study sites. Error bars indicate standard deviation (+1SD)

The seasonal change of phenolics in soil is presented in Fig. 6. From figure, it can be seen the the phenolics content of soil under kudzu stand was recorded in winter when there was no canopy was found, though the stem was over wintering belowground. At the onset of winter, kudzu plants starts senescing and sheds leaves. Kudzu produces very large amounts of active isoflavones, namely, genistein, daidzein and puerarin (Kirakosyan et al. 2003), and other phenolics like tannins and coumarins (Parks et al. 2002). These naturally active secondary metabolites can be released into the soil environment either as exudates from living plant tissues or by decomposition and leaching from plant residues (Putnam and Tang 1986). In one of our unpublished research, we have observed that kudzu leaves under decomposition release phenolics compounds into the soil. Therefore, it was likely that kudzu litter decomposition was responsible for the high concentration of phenolics in soil in winter. As spring approaches, the phenolics content of soil decreases and it start increasing when kudzu reaches the active growth phase in summer. It is believed that plants with high concentrations of phenolics can release them through root into the soil (Putnam and Tang 1986). Perhaps this is the reason behind the increasing content of phenolics with the growth of the plant.

From our study, we wanted to know whether the phenolics interact with soil elements. Table 2 provides the relation between soil phenolics and soil elements examined. We found that only TC in soil had negative correlation with soil phenolics, though the relation was not significant. All other elements measured in soil had positive correlation with soil phenolics. Only Cu, Zn and Na had significant positive correlation with the phenolic status of soil. Batish et al. (2002) found significant positive correlation with soil phenolics. Unlikely to our results, they found negative correlation with Zn and N.

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