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RESEARCH ARTICLE

Effects of nutrient concentration and litter cover on quantitative shoot parameters and belowground biomass of *Zizania latifolia* (L)

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This study investigated the effect of nutrient concentration and litter cover on the development of quantitative shoot parameters and belowground biomass (BGB) production of *Zizania latifolia*. *Zizania latifolia* is a common emergent aquatic species in East Asia. Four treatments were done at the study site, and were observed between May 2003 and December 2005. The treatments are namely, high nutrient (HN) with litter cover (HNWL), high nutrient without litter cover (HNNL), low nutrient (LN) with litter cover (LNWL), and low nutrient without litter cover (LNNL). The quantitative shoot parameters and BGB had higher values for treatments with high nutrient (HN) compared to the low nutrient treatments (LN), independent of the presence of litter cover. Furthermore, the life span of the secondary shoots was also higher in HN treatments compared to LN treatments. The BGB productivity was higher in the HNNL treatment compared to the other treatments. The LNWL treatment showed the least developed quantitative shoot parameters, e.g. plant height, and the lowest BGB for *Z. latifolia*. It was generally observed that the combined effects of low nutrients and litter cover negatively affected shoot development and BGB production.

Keywords: biomass; litter cover; nutrient concentration; quantitative; Zizania latifolia

1. Introduction

Litter is of recognisable importance in the growth and productivity of plants when they decompose into humus, thus providing nutrient to the plants [1]. In the natural environment, the litter layer is composed of leaves, flowers, fruits, stems, plant debris and also a smaller proportion of animal residue covering the soil surface. Accumulation of litter depends on factors such as the community primary productivity, as well as changes in weather, with rainfall being the main component [2,3].

The amount of litter accumulated at a site can be altered by the influx of litter from other sites, or by efflux to other sites. The micro–environment near litter affects its decay, thus changing its accumulation [1]. It has been reported that litter properties depict tremendous influence on soil nitrogen (N) cycling [4–6]. In their findings, Walker et al. [5] and Pastor and Walker [6], described the mechanism under which low nutrients and litter cover affected shoot development and BGB production of wild rice (*Zizania palustris L*.)

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Schweitzer et al. [7] reported that plant species could influence nutrient cycling through differences in litter quality, specifically those containing polymeric composition and polymer to nitrogen ratios. The amount and quality of plant litter input in the soil also has a strong impact on carbon (C) and nutrient cycling [8–10].

Zizania latifolia (Griseb.) Turcz. Ex Stapf (Z. latifolia) is one of the emergent aquatic species in East Asia, growing along the littoral zones of freshwater marshes and streams, and it can attain a maximum height of 2.6 m. Zizania latifolia has the ability to tolerate anoxic sediments and is usually found in deep habitats because its rhizomes have high ventilation efficiency [11–13].

Litter cover may hinder the penetration of light from reaching the struggling young shoots that emerge from the rhizomes of *Z. latifolia*. Previous studies on the effect of litter on grassland productivity, have reported that accumulation of herbaceous detritus limited production of C_4 grasses in established ecosystems [14,15]. These findings imply that the effect of litter on young shoots may differ from its effect on the productivity of established grasslands.

It is not surprising that litter can have far-reaching effects on plant productivity, because the litter layer changes micro-habitat properties (i.e. the quantity of solar radiation into the soil, soil chemistry, and soil moisture) and plant community interactions [14,16]. It is also possible that the accumulation of other types of litter (i.e. juniper and deciduous litter fall) may likewise decrease the competitive ability of grasses [16]. The inadequate light distribution due to litter cover may result in weakened plant individuals, which in turn causes low productivity [17], thus forcing the plants to morphologically adapt [18–20].

In the above regard, this study focused on the effect of nutrient concentration on the growth of *Z. latifolia*, the effect of litter on the growth of young *Z. latifolia* shoots, and the combined effect of both factors on the growth and productivity of *Z. latifolia*. Also, some studies have been conducted in the natural environment [10]. This experimental study is the first of its kind that combines the synchronous effect of the two factors in question.

2. Materials and methods

2.1. Experimental setup

The experiment was carried out at Saitama University, Saitama city (Japan), from May 2003 to December 2005. The size of the experimental plot was 2 m wide, 3 m long and 0.5 m deep, and was exposed to solar radiation. During the three growth seasons (i.e. May–December 2003, March–December 2004, and March–December 2005), the experimental plot was divided into two treatments, i.e. high nutrient (HN) and low nutrient (LN) treatments (the size of each being 1 m wide, 3 m long and 0.5 m deep).

Hyponex nutritional product was used as a nutrient enriching reagent for the soil. It is a suborganic product, which contains nitrogen (N), phosphorus (P) and potassium (K) in a ratio of 6:10:5, respectively. The total phosphorus (TP) concentration in pore water was kept constant, at approximately 0.52 ± 0.19 (mg/l) and 0.08 ± 0.02 (mg/l) for HN and LN treatments, respectively. The same was done for the total nitrogen (TN) concentration, which was kept at 7.29 ± 1.45 (mg/l) and 2.18 ± 0.38 (mg/l) for HN and LN treatments, respectively.

Prior to the start of the growth season in February 2003, *Z. latifolia* rhizomes containing young shoots were taken from a 5-hectare freshwater eutrophic marsh with homogenous stands in Hasuda city, Saitama prefecture, located 40 km north of Tokyo, Japan (35°59′ N, 139°40′ E), and were planted in the experimental plot. The young shoots which were planted were taken from one population, in order to ensure homogeneity. Details of the parent eutrophic marsh are described by Lan et al. [21].

Water depth was kept constant, i.e. approximately 30 cm throughout the entire period, and was replenished twice weekly, to avoid shading effects resulting from phytoplankton blooming. It should be noted that no treatment (i.e. addition of nutrients) was performed on the experiment during the first four weeks after commencement. This was done to ensure that the plants acclimatise to their new environment. The aboveground parts of *Z. latifolia* grew from March (the start of the growing season) and died off in December.

In order to assess the influence of litter cover on the growth and productivity of the plants, two thirds of each experimental treatment was covered with *Z. latifolia* litter material. This was done at the beginning of the second growth season. Thereafter, at the end of each growth season, the litter from two thirds of each treatment was retained. In addition, litter from the other third was also transferred and added to it. This was done for the last two growth seasons. In total, the experiment contained four treatments, i.e. high nutrient with litter (HNWL), high nutrient without litter (HNNL), low nutrient with litter (LNWL) and low nutrient without litter (LNNL).

The amount of litter cover used in the second growth season of the experiment was 1891.76 ± 27.40 g DW and 17.00 ± 0.88 cm thick, for every square meter of surface area, while that of the third growth season was 1905.87 ± 33.37 g DW and 17.58 ± 0.34 cm thick, per square meter.

During the first growth season, all litter cover conditions were made identical. The results of the first season were also plotted in the Figures (e.g. Figure 4). This was done to ensure easier one-glance comparisons with the subsequent seasons, on the effects of nutrient concentration and litter cover on the growth and production of *Z. latifolia*.

2.2. Pore-water analysis

Twelve pore-water samples (six from each treatment, HN and LN) were taken once a month for TP and TN analysis, using the Hounslow [22] procedure of taking samples for nutrient analysis. The sampling followed the US Environmental Protection Agency (EPA) system. All pore-water samples were filtered through a glass micro-fibre filter (GFC, Whatman, Australia). Thereafter, the samples were stored at -20 °C for one week in the laboratory. TP and TN concentrations were analysed as stipulated by APHA et al. [23].

Furthermore, at the beginning and end of the four-week acclimatisation period, the total phosphorus (TP) and total nitrogen (TN) of the pore water from the experimental plots was analysed. This analysis was done once a month thereafter, throughout the entire study period.

2.3. Quantitative shoot parameters and belowground biomass (BGB) measurements

Surveys of quantitative shoot parameters of the plants (both primary and secondary shoots), i.e. number of shoots, shoot diameter and shoot height, were measured and recorded twice every month, throughout the entire experimental period. This was done for all the shoots in the plot. The positions of the plants were marked and recorded using the x-y coordinate grid reference, taken along two adjacent peripheries perpendicular to each other, using a tape measure. This marking was done for purposes of future measurements and identification of the shoot parameters.

For BGB analysis, three replicate plant samples (25 cm by 50 cm, up to a soil depth of about 20 cm) were taken from each treatment, at the end of each growth season. The live belowground parts were separated into base stem, roots, fresh, yellow and hard rhizomes. The belowground parts were then oven dried to dry weight at 85 °C for 48 h, to determine their respective biomass.

2.4. Data analyses

All statistical analyses, particularly two-way analysis of variance (ANOVA, multiple comparison (*post-hoc*) analysis of the mean, also see Table 1) and *t*-tests were performed by using SPSS

	Treatment	Sum of squares	df	Mean square	F	<i>p</i> -value
Primary shoot density	HNNL	581723.970	56	18931.580	12.368	0.000
	HNWL	432168.775	56	13480.274	117.952	0.000
	LNNL	365401.975	56	12505.394	45.548	0.000
	LNWL	343715.086	56	10748.796	49.332	0.000
Shoot height	HNNL	347375.328	56	7551.638	96.858	0.000
	HNWL	339915.073	56	7081.564	129.447	0.000
	LNNL	275648.831	56	5992.366	144.256	0.000
	LNWL	274386.565	56	5515.882	98.278	0.000
Shoot diameter	HNNL	1859.768	56	41.328	32.821	0.000
	HNWL	1698.437	56	36.137	25.668	0.000
	LNNL	1455.485	56	27.512	74.783	0.000
	LNWL	1161.107	56	25.145	22.475	0.000
BGB	HNNL	186.601	4	4.665	22.346	0.000
	HNWL	183.893	4	4.378	72.225	0.000
	LNNL	153.916	4	3.665	27.372	0.000
	LNWL	174.742	4	3.974	59.998	0.000

Table 1. ANOVA* table of results showing differences between the four experimental treatments for the different measured parameters. HNWL, high nutrient (HN) with litter cover; HNNL, high nutrient without litter cover; LNWL, low nutrient (LN) with litter cover; LNNL, low nutrient without litter cover; BGB, belowground biomass.

*Multiple comparison (*post-hoc*) analysis was conducted for all ANOVA results which were significant at p < 0.05, and depicted significant differences for all the four treatments.

version 12.0 for Microsoft Windows (dated September 2003, SPSS Inc., 1989–2003) to determine seasonal variations in quantitative shoot parameters and BGB, and to compare them in both the HN and LN experimental treatments.

3. Results

3.1. Variation of quantitative shoot parameters of Z. latifolia

During monthly assessments from March to December for the 3-year duration, it was observed that most of the dead shoots were found around the centre of each treatment system. The variations were clearly evident in the second and third growth seasons of the experiment. Shoot emergence of *Z. latifolia* was observed twice every month. The number of shoots for each treatment is shown in Figure 1. In addition, during the period between August and October, a number of secondary shoots emerged from lateral buds, especially from long horizontal rhizomes of the primary shoots. Secondary and primary shoots in all treatments survived up to early December.

The primary shoot density started rising in early May for the treatments without litter, while for the treatments with litter, the rise began in mid May, and then both gradually dropped until November. This showed that the longest survival period of shoots was between early February and December for HNNL and LNNL treatments compared to HNWL and LNWL treatments, whose primary shoots emerged two weeks after the sprouting of the former.

The percentage survival rate of the primary shoots in December was 16.62 % for the HN treatment and 8.05 % for the LN treatment. The density of primary and secondary shoots was higher in HN treatment compared to LN treatment (Figure 1). The seasonal trend of shoot parameters at the experimental plot corresponded well with that of the parent marsh in Hasuda City [21].

Furthermore, the amount of secondary shoots, which emerged between August and late October was higher in the treatments that were litter free, compared to the littered ones. It was 43.86 % higher in HNNL compared to HNWL, and 37.52 % in LNNL compared to LNWL, for about 2



Figure 1. The mean primary shoot densities (\pm SE) in the four treatments, during the three years of the experimental period (from May 2003 to December 2005).



Figure 2. The mean shoot heights (\pm SE) of the four treatments, during the three years of the experimental period (from May 2003 to December 2005).

months (i.e. between August and October). Thus, the number of secondary shoots was significantly higher in HNNL treatment compared to the rest (Table 1, ANOVA results).

On the other hand, the primary shoots in HN and LN treatments increased steadily in height and peaked during early September (Figure 2). Thereafter, a decline was observed till the end of the growth season. On average, the shoot heights of HN treatments were significantly taller than those of LN treatments (*t*-test, p < 0.05, and Table 1). Conversely, the difference in shoot heights between the treatments covered with litter, compared to those without litter, was insignificant (HNNL and LNNL) (*t*-test, p > 0.05, and Figure 2).

The average diameter of primary shoots for each treatment is depicted in Figure 3. The average shoot diameter steadily increased and attained its maximum girth in late August for HN treatments (e.g. in 2004, 17.35 \pm 4.76 mm for HNNL and 16.80 \pm 3.82 mm for HNWL), late August for



Figure 3. The mean (\pm SE) primary shoot diameters in the four treatments, during the three years of the experimental period (from May 2003 to December 2005).

LNNL, and early September for LNWL treatments, respectively (e.g. in 2004, 13.84 ± 3.26 mm for LNNL and 12.94 ± 3.06 mm for LNWL).

3.2. Variation of BGB

The BGB was relatively higher in the HN treatments compared to the LN treatments. Consequently, BGB was lower in the litter-covered treatments compared to those without litter. This resulted into a quadratic relationship between litter biomass and total biomass of plants in HNWL. A comparison



Figure 4. The variation of belowground biomass in the four treatments at the end of each growth season (HNWL, High nutrient (HN) with litter cover; HNNL, high nutrient without litter cover; LNWL, low nutrient (LN) with litter cover; LNNL, low nutrient without litter cover), during the three years of the experimental period (from May 2003 to December 2005).

of BGB among the treatments depicted that HNNL had the highest BGB whereas LNWL had the lowest (Figure 4). This gave correlations for the relationship between nutrient concentration and BGB (e.g. $R^2 = 0.817$; p < 0.05 in the HN treatment).

Statistical analysis showed a significant combined effect of nutrient concentration and litter cover on the plant BGB (Table 1). The comparison of HNNL with HNWL showed that production and quantitative shoot development was higher in the former, compared to the latter. However, the difference was not statistically significant (*t*-test, p > 0.05), as it was in the scenario with different nutrient treatments (Table 1).

4. Discussion

Both the quantitative shoot parameters and BGB of the plants varied significantly in all the four treatments, i.e. HNWL, HNNL, LNWL and LNNL (see Table 1), implying that nutrient and litter cover had a significant impact on BGB and quantitative shoot parameters of *Z. latifolia*.

Nutrient availability for the plants is usually a limiting factor for the development of their organs, and subsequently primary productivity [4,5]. Thus, the study showed that HN treatments had relatively higher productivity (e.g. Figure 4). Also, if external factors such as light, temperature, water, etc are influenced by litter cover, similar effects on plant growth occur [1,15]. Our study showed that litter cover physically affected the early emergence of young shoots, plus a delay in the shoot density peaks (Figure 1). These findings are similar to those reported elsewhere [2,5,6,14].

In addition, litter cover reduces soil temperature amplitude and water evaporation, thus increasing local humidity [16]. On the other hand, sometimes litter reduces rainfall run off infiltration into the soil. Also, litter acts as light filter inhibiting the germination of light-sensitive young shoots, which are in dire need of light for further growth to take place [24,25].

The decay of litter affects the chemical properties of the soil through release of phyto–toxic substances, which inhibit root growth, plus shoot emergence and development [26]. However, this also increases the availability of nutrients in the soil, thus influencing shoot elongation and growth [6,26]. It has also been reported that recruitment is strongly affected by the presence of litter, due to their dependence on the surrounding microhabitat for survival [24,27,28].

Hager [29] reported that plants and plant litter had important effects on plant colonisation, plus community composition by affecting both young and senile shoot survival and growth. In that study, the *Lythrum salicaria* shoots significantly increased in growth following the removal of both *Typha* plants and litter in drier wetlands. Similar trends were observed in our study, productivity of NL treatments was significantly higher than that of WL treatments (Figure 4, Table 1). Non-decomposed plant litter pose a negative impact on emerging shoots, since they alter micro-environmental factors like solar radiation penetration and soil moisture [1].

Furthermore, treatments with low nutrient concentration and litter cover depicted poorly developed structures, i.e. the girth size of shoots and shoot densities, etc. (Figures 1 and 3). This might be due to the shortening of leaves, thus resulting into poor development in other plant organs [5,10]. In their findings, Walker et al. [5] and Pastor and Walker [6], described the mechanism under which low nutrients and litter cover affected shoot development and BGB production of *Zizania palustris L*. The absence of litter cover in the HNNL treatment enabled the development of denser, taller, thicker shoots [5,14,29], and relatively higher BGB [1]. In the LNWL treatment, the plants contributed little in depositing organic matter [29], due to the low production they depicted [5] (Figure 4).

Thus, litter cover alone did not significantly affect the study results, but nutrient concentration also played a role [5,6,9,24]. The study observed a significant difference in shoot density for LN

treatments. The results implied that because of litter cover, the LNWL treatment had lower shoot density compared to LNNL, clearly vindicating that nutrient concentration was not the only factor influencing growth of *Z. latifolia* [5,9]. In general, the results suggested that the presence of litter hindered the emergence and growth of shoots [1,24].

In studies of litter cover and decomposition [30,31], litter loss increased with temperature. In the present study, both the quantitative shoot parameters and BGB of the plants varied significantly in all the treatments, implying that nutrient and litter cover had a significant impact on BGB and quantitative shoot parameters of *Z. latifolia*. The ecological significance of our findings imply that litter cover inhibits plant production [5,6], because it affects the micro-habitat properties of soil (the quantity of solar radiation into the soil, soil chemistry, moisture, etc.), and plant community interactions [14,16]. Subsequently, the inadequacy of these resources like light may result into weak plant individuals [17].

On the other hand, the presence of litter cover can influence soil nitrogen (N) cycling [4–6]. High values of both soil moisture [32–34] and temperature [35] can favour litter decomposition. Furthermore, alterations of water bodies such as hydrological disruption by dams have both direct impacts on riparian vegetation [36] and reduce the total litter deposition, thus reducing litter cover on the soil [30,31].

Microbial nutrient demand has also been reported as a factor that influences the BGB and quantitative shoot parameters, in litter cover studies [5]. However, we did not investigate this factor; neither did we measure the nutrient content of BGB. Consequently, these are some of the shortcomings of our study.

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