Dynamics of growth, carbon and nutrient translocation in *Zizania latifolia*

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Abstract

We studied the seasonal resource dynamics between organs of wild rice (*Zizania latifolia* (Griseb.) Turcz. ex Stapf.) to obtain a better understanding of its growth dynamics, carbon and nutrient translocation. The results of observation from January 2002 to February 2004 showed the shoot density markedly increased after emergence of shoots at the end of March until May (up to 800 ind/m^2). However the shoot mortality due to self-thinning reduced the total new shoots by more than 70% by the end of July. Thereafter, the shoot density was nearly constant with the aboveground biomass peaking at the end of August. In the late winter, the rhizome biomass declined by respiration loss to about 25% of its peak value. Meanwhile the decline in rhizome reserves from January to the end of April was about 20%. This small reduction compared with other perennial emergent species implies that there is a lower contribution of rhizome reserves to support new shoot formation. The initial heterotrophic growth of new shoots based on the rhizome resources lasted for a short period, then switched to autotrophic growth at the end of April or the beginning of May. Thus, in most periods of foliage development, nutrients were obtained mostly from soil through uptake by roots, not through resource allocation of the rhizome. In autumn, the standing dead shoots retained most of the nutrients and carbohydrates without translocating downwards. This suggests that in practice, the plant can remove nutrients from sediment more efficiently than other emergent plants.

Key words: *Zizania latifolia,* carbon cycle, emergent macrophytes, nutrient cycle, self-thinning

Introduction

A wild rice species, *Zizania latifolia* (Griseb.) Turcz. ex Stapf., is one of the most common emergent species in East Asia, occurring along the littorals of freshwater marshes and streams (Okuda, 1978). With its high nutrient absorption capacity, *Z. latifolia* has great potential as a species for wastewater treatment wetlands (Miyata, 1993) or as a species to restore stream or littoral ecosystems (Okuda, 1978), where the common reed (*Phragmites australis*) has usually been used. Although its leafy and dense shoots are sometimes viewed as a potential threat to river management, its flexible structure makes it possible to create a vegetation area even under intense mechanical disturbances (Asaeda et al., 2005a). Moreover, *Z. latifolia* is one of flood-tolerant crops and Bruins et al. (1998) has proposed a solution by converting rice to flood-tolerant crops such as *Z. latifolia* for lowland areas where flooding has often caused the crop loss

Compared with *P. australis*, studies on the seasonal pattern of *Zizania* spp. have been limited (Lee and Steward, 1981; Yamasaki and Tange, 1981; Weiner and Whigham, 1988; Power, 1996). Tsuchiya et al. (1993) reported the annual dynamics of *Z. latifolia* including the formation of secondary shoots, life span of leaves, and annual biomass variation. Relatively low net production was also reported (Tsuchiya et al., 1997). In addition, the spring mortality of *Zizania* spp. shoots was intensively studied by Weiner and Wigham (1988), yet the discussion on the nutrient cycles and their survival strategies was very limited when compared with *P. australis* (Kuhl et al., 1997; Lippert et al., 1999; Asaeda et al., 2005b).

Like *P. australis*, *Z. latifolia* is a rhizomatous perennial plant that depends on its rhizome system for survival and expansion of colonies (Chapin et al., 1990). However, its relatively low root/shoot biomass ratio compared with *P. australis* (Tsuchiya et al., 1993) suggests that the plant has more efficient resource translocation and less dependence on rhizome resource, although further studies are necessary on carbohydrate budgets between the above- and belowground organs. With *P. australis*, standing stocks of nonstructural carbohydrates and mineral nutrients in rhizomes decrease to one-third during the heterotrophic growing stage of foliage, then increase towards the end of the growing season by downward translocation (Dykyjova and Hradecka, 1976; Schierup, 1978; Graneli et al., 1992; Asaeda et al., 2006b).

Although *Z. latifolia* is one of the most important members in the littoral zone of streams and rivers, the seasonal resource dynamics between organs of the plant is still unclear due to limited available information. This study was aimed at elucidating the

phenology by examining the dynamics of growth and carbon, nitrogen and phosphorus allocation in the above- and belowground compartments of wild rice.

Study Site

The study site was a uniform stand of *Z. latifolia* about 30 m by 30 m wide at the fringe of a freshwater marsh 40 km north of Tokyo (35º59′00″N, 139º40′53″E). In 2002, the marsh was muddy and inundated throughout the year, except in January-March and December. In 2003, the marsh was dry from January to May, inundated to about 10 cm depth in June and July but dry again from August to September. From October to November, the marsh was inundated again up to 30 cm depth, however, the water level started to decrease in December and dry during winter. A *P. australis* stand had been present adjacent to the *Z. latifolia* colony for more than 15 years, and during the inundation period, water depth and substrate conditions were essentially the same between the colonies. Observation was conducted once a month or once every 6 weeks from January 2002 to February 2004. Daily temperature, solar radiation and precipitation recorded at the nearby weather observatory (10 km south) indicated that meteorological conditions during the observation period were normal in this area. Located in the center of natural reserves, nutrient concentration in the water column was relatively stable during the observation period; 1.08–1.8 mg/l for total nitrogen, 0.04–0.3 mg/l for ammonium nitrogen, and 0.013–0.27 mg/l for total phosphorus.

Methods

Biomass sampling and chemical analysis

At each sampling, three replicates of above- and belowground biomass were sampled in three quadrats 50 cm by 25 cm wide, taken in a uniform part of the stands, more than 2 m away from the edge of the stands and from previously sampled spots. Belowground biomass was excavated as a 50 cm by 25 cm wide and about 40 cm thick undisturbed soil block, and all remaining roots and rhizomes at the bottom of the hole were also collected. The belowground samples were carefully washed in a 4-mm mesh sieve by pressurized tap water and all materials retained in the sieve were collected for further sorting. The aboveground parts were sorted into live and dead shoots. Brownish parts of shoots were also separated and were sorted into dead shoots. Then, the living shoots were sorted into stems, leaf sheaths and leaf blades. Shoot density, shoot height, and the number of live and dead leaves were recorded for both living and dead shoots. Living belowground parts

were sorted first into rhizomes, stem bases, roots and buds. Foul-smelling and non-turgid materials were considered as dead. Rhizomes were distinctively categorized into two groups, i.e. less than and more than 7 mm in diameter. The thinner rhizomes extended vertically from the stem base, spreading widely after turning horizontally, while the thicker rhizomes extended relatively horizontally. Rhizomes were classified into four categories, white fresh, yellow hard, yellow soft rhizomes and vertical rhizomes, based on their textures, colors, hardness, smells, and morphologies. Mortality loss of rhizomes during the period of two consecutive observation times was estimated from the amount of dying rhizomes at the start of the period. Although this does not correspond exactly to the mortality during the period, the comparison with the increment length of dead rhizomes in the period indicated a favorable agreement (R^2=0.66). A small portion of each sorted fresh sample was freeze-dried, weighed and ground into powder with a Wiley Mill for chemical analyses. The remaining portion was dried at 85ºC for more than 72 h until constant weight was achieved.

Analyses of total nonstructural carbohydrates (TNC), water soluble carbohydrates (WSC), total carbon content (TC), total nitrogen (TN) and total phosphorus (TP) were performed in triplicate, separately for each rhizome category, roots, stem bases, leaf blades, sheaths and stems, and dead rhizomes and shoots, respectively. Both TC and TN were determined with a CHN-analyzer (MT-5, Yanaco CHN corder, Japan). The TP was determined by the Molybdenum Blue method (Murphy and Riley, 1962). The TNC, a measure of the total energy reserves, was determined using α-Amylase (EC 3.2.1.1 Type VII-A, Sigma–Aldrich Co., USA), based on a procedure developed by Wong (1990). The WSC, immediately translocatable carbohydrates (Granelli et al., 1992), was extracted with hot water. In all cases, carbohydrates in the extracted solutions were measured by the phenol–sulfuric acid colorimetric procedure of Dubois et al. (1956). Standards of starch and sugars, as well as plant powder standards, were used as controls throughout the analysis. The total carbon concentration of the structural component (TSC) was estimated as the TC concentration minus the carbon concentration of the TNC, containing carbon at approximately 42%, based on analyses of pure starch spiked with 0–20% glucose using the CHN-analyzer. The standing stock of each component in individual organs was then estimated as the concentration per unit area. Then, the standing stock in each category was calculated as the product of concentration and biomass.

The respiration rate of each rhizome category was obtained at 20ºC, following Cizkova and Bauer (1998). Then, it was converted to the value of the temperature at each time by multiplying by θ^{T-20}, where θ is the Arrhenius constant (=1.08) and T is the

monthly average temperature (Bitton, 1998). Solar radiation inside the canopy was measured with an illuminometer (PCL-01L, PREDE Co. Ltd., Japan) at several points every 25 cm in height together with the shoot biomass above that level.

Carbon fluxes and pools in the above- and belowground compartments

Carbon budgets transferred between above- and belowground organs and the synthesis rate of structural components were estimated from the conservation of carbon. The total carbon stock of the belowground system is enhanced through downward translocation from aboveground components, although a part of it is lost by the mortality and respiration losses of plant organs. Therefore, the translocation rate of carbon can be described by:

$$TR(t) = \frac{dC_B}{dt} + \frac{dDC_B}{dt} + RL(t) \quad (1)$$

where $TR(t)$ is the translocation rate between the above- and belowground systems, C_B is the standing stock of total carbon in the belowground biomass, DC_B is the total carbon stock in the dead belowground organs (the dead biomass multiplied by its total carbon concentration), and $RL(t)$ is the belowground respiration loss.

Thus, the net production rate in the aboveground system, $NP(t)$, can be obtained as the sum of the increment of the aboveground total carbon stock, the amount of carbon left in the dead biomass, and the amount translocated downwards, i.e.

$$NP(t) = \frac{dC_A}{dt} + \frac{dDC_A}{dt} + TR(t) \quad (2)$$

where $NP(t)$ is the net production rate in the aboveground system (photosynthesis rate minus metabolic loss), C_A is the standing stock of total carbon in the aboveground biomass, and DC_A is the total carbon stock of the dead aboveground organs. (Note: downward translocation was defined as positive)

The synthesis rate of the total structural components is given by,

for aboveground:

$$SC_A(t) = \frac{dTSC_A}{dt} + \frac{dDTSC_A}{dt} \quad (3)$$

for belowground:

$$SC_B(t) = \frac{dTSC_B}{dt} + \frac{dDTSC_B}{dt} \quad (4)$$

where $SC_A(t)$ and $SC_B(t)$ are the synthesis rates of the structural components for above- and belowground systems, respectively. TSC_A and TSC_B are the total structural carbon stocks in the living above- and belowground systems, respectively, and $DTSC_A$ and

$DTSC_B$ are the structural carbon stocks in the above- and belowground dead tissues, respectively.

The daily net increment of the standing stock between two consecutive observation times in each category was obtained as the differentiation with respect to time.

Nitrogen and phosphorus fluxes and pools in the above- and belowground compartments

Different from carbon, uptake of nitrogen and phosphorus is from substrates. Thus, the daily aboveground translocation rates are

$$TRN = \frac{dN_A}{dt} + \frac{dDN_A}{dt} \quad (5)$$

Where TRN is the upward translocation rate to shoots, N_A is the total amount in shoots, DN_A is the total amounts in dead shoots. Thus, dDN_A/dt indicates the rate of loss due to shoot mortality.

Then, the net uptake rate from the substrates is

$$UN = \frac{dN_B}{dt} + \frac{dDN_B}{dt} + TRN \quad (6)$$

Where UN is the net uptake rate from the substrates, N_B is the total amount in the belowground organs, dDN_B/dt is the rate of loss due to mortality of the belowground organs.

Results

Seasonal variation of shoot density and morphology

Observation was conducted from January 2002 to February 2004. Figure 1 (a) and (b) show the shoot density and the average shoot height at each observation time and the number of living and dead leaves per shoot. The maximum of the shoot density in early spring was 25% higher in 2002 than in 2003, probably because of the higher early spring temperature in 2002 (Figure 2). However, the trends of seasonal variation were essentially similar between the two years. Almost all new shoots were reproduced vegetatively by

emerging from the stem bases of the previous year. The shoot density markedly increased after the start of emergence of shoots at the end of March until May, i.e. up to about 800 ind/m^2 in 2002 and 560 ind/m^2 in 2003, and then gradually decreased down to about 150 ind/m^2 at the end of July, thereafter stabilizing with nearly constant density. Only a small number of shoots emerged secondarily. All aboveground biomass died by the middle of December.

The average shoot height gradually increased up to 250 cm by late July, then maintained constant height thereafter. In spite of their 2 weeks earlier emergence than the *P. australis* in the adjacent stand, the average height of *Z. latifolia* shoots was overtaken in the middle of May by the fast-growing *P. australis*. At the end of May, the average shoot height of *Z. latifolia* was about 40 cm lower than that of *P. australis,* based on direct field measurement and no *P. australis* biomass was sampled. The average number of living leaves per shoot of *Z.latifolia* constantly increased up to 6.5/shoot until senescence at the end of August, followed by the increasing number of dead leaves at about 80 days behind, indicating 80 days for the life period of the leaves. Meanwhile, the life span of leaves reported by Tsuchiya et al. (1997) was 25% shorter.

Seasonal variation of biomass

Figure 3 (a) and (b) show the dry weight of above- and belowground biomass organs. The shoot biomass increased from the end of March, which was associated with the emergence of new shoots until the end of April and the growth of each shoot thereafter until August. The shoot biomass peaked at 2100 g/m^2 in 2002 and 1740 g/m^2 in 2003 at the end of August. The difference is perhaps due to the warmer spring temperature in 2002 and slightly lower water depth. Although the peak biomass was 20% different between the 2 years, the seasonal patterns were essentially identical. The dead shoot biomass increased gradually from the end of May to the end of July, and then declined slightly in August before markedly increasing due to senescence of shoots from October to December.

The total belowground biomass declined from the end of March to April to 370 g/m^2 in 2002 and 350 g/m^2 in 2003, but then substantially increased to 1800 g/m^2 in 2002 and 1550 g/m^2 in 2003 at the end of September, which was a month after the aboveground biomass peak in August. In winter, the belowground biomass constantly declined at about 3.4 g/m^2 day. Although some differences existed in the observed belowground biomass between 2002 and 2003, the patterns were also similar and the mean for each category between 2002 and 2003 were not significantly different.

White fresh rhizomes that appeared from the end of May, gradually changed to yellow, their biomass markedly increased in August, then declined after October because some of them had turned into the yellow hard category. Rhizomes formed in the latter half of the year changed to yellow hard rhizomes during the winter, and slightly increased until the end of July. From the middle of the previous year until winter, they turned into the dark yellow soft category and mostly died off through winter respiration and by translocating reserves to form new shoots in the next spring (Westlake, 1982). The stem base biomass declined from the end of March to the end of April, then began to increase again in May, along with the growth of shoots. In September, the stem base biomass accounted for about one third of the entire belowground biomass. The R/S ratio (the ratio of the belowground to the aboveground biomass) was smallest in September at about 0.85.

Carbon fluxes and pools in the above- and belowground compartments

Figure 4 (a) to (c) provide the component budgets of TC, the downward translocation of carbohydrates compared with the daily net production in the aboveground organs, and the daily synthesis of TSC in the above and belowground biomass. The daily budgets of TC, TNC, and TSC in the above- and belowground systems had similar patterns in both 2002 and 2003.

Figure 4 (a) shows that the net carbon production by shoots started to increase from March, peaking at the beginning of August, at 28 g C/m^2 day in 2002 and 20 g C/m^2 day in 2003, then declined to almost zero in October. The depression at the end of August 2002 was likely because insufficient replication and rapid losses of the dead standing shoot biomass caused by heavy rainfall and strong wind. As the precise amount of dead standing biomass losses could not be determined, the net production for this period was probably underestimated. Meanwhile, the rhizome respiration loss gradually increased from June to August and then gradually declined afterwards until November, associated with the increasing rhizome biomass and rising temperature. The rhizome respiration loss peaked between August and September and it accounted up to about one-quarter of the net production.

In spite of some differences between 2002 and 2003, carbohydrates were continuously translocated downwards from April (in 2002) or May (in 2003), that is about one or two months after the beginning of the growing season. The highest downward translocation occurred in August at 10–17 g C/m^2 day, and lasted until November (Figure 4 (b)). The downward translocation accounted for about 20–60% of the net production

until August, while from August to September almost 100% of the net production was mobilized into the rhizome system. Small negative values, 0.5 to 1.5 g C/m^2 day of TNC flux, were observed in March before starting the downward translocation. This was likely linked to the amount of resource that was mobilized to form new shoots.

After the senescence of shoots, until the emergence of new shoots in March, the rhizome TNC stock substantially declined due to respiration loss, while there was only a slight reduction in TSC, by mortality. Carbohydrates were translocated to the rhizomes throughout the growing season except for the initial short period of shoot growth and yet the structural components in rhizomes were synthesized mainly in two periods, i.e. May, and August to September (Figure 4 (c)). These periods corresponded to the peaks in downward translocation, i.e. due to the intense mortality of shoots in spring and the beginning of senescence of shoots in autumn. After the senescence of the aboveground organs, until the emergence of shoots in the following March, the rhizome biomass constantly declined, attributed to the loss of TNC rather than conversion from TSC. The balance between TNC reduction and metabolic loss during the winter apparently indicates that the metabolism consumes only rhizome TNC rather than TSC.

Uptake of nitrogen and phosphorus

Figure 5 (a) and (b) show the net uptake of nitrogen and phosphorus by above- and belowground organs. In spite of the slight difference between 2002 and 2003, the same seasonal pattern was observed for the net nutrient uptake rates. Although uptake by roots began relatively early in March or the beginning of April, i.e. after the shoots started growing, rhizome stocks were further used for 1 or 2 months until May. Then the uptake rate increased until August, up to 0.80 g N/m^2 day and 0.085 g P/m^2 day in 2002, and 0.40 g N/m^2 day and 0.042 g P/m^2 day in 2003. Because of the intense shoot mortality from May to July, substantial amounts of nitrogen and phosphorus, 0.14–0.2 g N/m^2 day and 0.014–0.028 g P/m^2 day, were released into the environment. Although the number of dying shoots declined gradually in the period, the daily nutrient effluent enhanced along with the growth of each shoot. Finally, it amounted to about half of the total daily uptake. The shoot growth and the intensive uptake of nitrogen and phosphorus by shoots ended in August followed by the remarkable growth of the belowground system in September. In October nitrogen and phosphorus were intensively translocated into the rhizomes; then from November to December, significant amounts of nitrogen and phosphorus were lost through shoot senescence.

Discussion

Zizania latifolia occurs normally under similar conditions to *P. australis*. However, in the littoral gradient of lakes, *Z. latifolia*, possessing a highly efficient ventilation system, occupies a deeper zone than *P.australis*, although its tolerance to reduced soil is less than *P. australis* (Yamazaki, 1984). However, in spite of their inferior morphology (Szczepanska, and Szczepanski, 1976) to *P. australis* in terms of reception of light, stable *Z. latifolia* colonies are also found in very shallow water, which otherwise is favorable for *P. australis*. Competition among the emergent species has been discussed from several points of view, e.g. water depth (Yamazaki and Tange, 1981; Grace and Wetzel, 1982; White and Ganf, 1998; Strand, 2002), pre-emption (Grace, 1987), and morphological characteristics (Szczepanska and Szczepanski, 1976). Although ventilation ability is a crucial factor for territorial distribution in the water gradient, other factors such as morphological characteristics or tolerance to the abundance or shortage of nutrients, also affect competition in shallow water.

Carbohydrate translocation in the heterotrophic growth stage of shoots

The amount of belowground TNC continuously declined in winter due to respiration loss, and the deficit increased only slightly if any after the initiation of shoot formation in March, likely due to the upward mobilization of carbohydrates to form new shoots. The decline in rhizome reserves was approximately 70 g C/m^2, which includes the upward translocation, leaching and respiration loss. This corresponds to 20% of the rhizome stock in January. Therefore, the reduction in rhizome stock was smaller for *Z. latifolia* compared with other perennial emergent species. For example, the declines of rhizome stocks to the total rhizome reserves in winter were reported as 21% (Schierup, 1978) and 25–30% (Graneli et al., 1992) for *P. australis*, 35% for *Typha* spp. (Garver et al., 1988) and 50% for *Typha orientalis* (Roberts and Ganf, 1986).

The belowground TNC budget turned to positive at the end of April or the beginning of May, due to the start of downward translocation of photosynthetic products, switching to the complete autotrophic growth of shoots from initial heterotrophic growth. Although this switching time depends on the meteorological conditions at that time of year, it was at least a month earlier than those of *P. australis* and *T.angustifolia* that were observed in the middle of June in the same area (Karunaratne et al., 2003; Asaeda et al., 2006a; Asaeda et al., 2006b). Because of this early switch, the heterotrophic growth of *Z. latifolia* new shoots was supported from the belowground TNC for only less than 2

342 months or 15% of the entire growing period. This heterotrophic growth stage is relatively
343 short compared with 40% (Fiala, 1976) and 60% (Schierup, 1978) for *P .communist*,
344 25–30% (Graneli, 1992) for *P. australis* and about 20% for *T. orientalis* (Roberts and
345 Ganf, 1986). Therefore, compared with *P. australis* and *Typha* spp., *Z. latifolia* depends
346 less on rhizome stocks to develop its foliage.
347

Effect of a large amount of shoot emergence and spring mortality on invasion of other species and nutrient cycling

About 500–800/m^2 of shoots emerged from March to May, which was several times larger than the approximately 80/m^2 shoot emergence of *P. australis* in the adjacent stand. However, the number declined significantly from the end of May and down to about 150/m^2 in August when the shoot growth ended. A similar behavior was also found for the same genus, *Z.aquatica* (Weiner and Whigham, 1988). The extremely large amount of shoot emergence is enabled by the small size of buds and new shoots, about 0.03 g each compared with 0.1–0.2 g each of *P. australis*, and relatively shallow positions of bud formation; less than 15 cm deep from the ground surface, compared with about 30 cm deep with *P. australis*. The large root system of *Z. latifolia* occupies a large subsurface space, which may inhibit other species from invading. However, it was observed that individual *P. australis* as well as many other annual species emerged in the *Z. latifolia* stand, especially if there was no litter, indicating that the root system alone is insufficient to block the invasion of other species. It was reported that the accumulated litter layer of *P. australis* efficiently interfered with other species' invasion by shading (Thompson and Shay, 1985; Graneli, 1989; Gryseels, 1989). Compared with *P. australis*, which has rigid stems and remains standing for several years after mortality (Hocking, 1989; Graneli, 1989) or *Typha* spp., which has relatively hard leaves, *Z. latifolia* leaves are soft and litter accumulation was recorded as only about 20 cm thick. However, the light attenuation decreased sharply and rapidly inside the litter layer, down to less than 5% of the global radiation at the level of the soil surface. Thus the litter layer can function effectively to prevent the invasion of low plants or the initial stage of other invasive plants, although not for large perennial plants, such as *P. australis*. There was no emergence of other species under the *Z. latifolia* litter layer, except for *P. australis*, which emerged 2 weeks later than *Z. latifolia*. A short period of heterotrophic growth stage of *Z. latifolia* could be a benefit from the small thickness of the litter layer, as it was observed that the shoots became taller than the thickness of the litter layer in the middle of April, which was also the period of switching from the heterotrophic to autotrophic growth stage.

Early emerged shoots with their small size have a high mortality risk; therefore, the large quantity of shoot emergence can be a measure to overcome this risk. Furthermore, early emergence of many shoots reduces the light intensity underneath the litter layer of *Z. latifolia*. As the temperature and light intensity on the ground surface fall, the emergence of other species in the area will be hampered. *Zizania latifolia*, which emerged 2 weeks earlier, was taller than *P. australis* until the middle of May. After that,

shoot density gradually regressed as the shoot height increased. Although this hypothesis has not been confirmed yet, it is consistent with the earlier emergence of more shoots after the removal of the litter layer, in order to interfere with the emergence of other species (Asaeda et al., 2005b).

Latter stage of shoot growth

Net production increased from April to August and was approximately 2500 g C/m^2, the average value for 2002 and 2003. About 32% was synthesized into aboveground structural components, while 61% was mobilized into the belowground organs. Of the 61% downward translocation, 20% was synthesized into structural carbohydrates to form new rhizomes, and 60% was consumed by rhizome respiration. The formation of new rhizomes took place between April and May, earlier than *P. australis*, which intensively creates new rhizomes from June in this area (Karunaratne et al., 2003; Asaeda et al., 2006a). Associated with the end of shoot growth in August, the synthesis of structural carbohydrates was basically terminated in the shoots. Then, all net production in the aboveground organs was translocated downwards; approximately 30% was synthesized into structural carbohydrates to support vigorous formation of new rhizomes and stem bases, and the remainder was mostly respired.

Shoots died mostly between October and November, while carbohydrates contained in the shoots mainly remained in the dead tissues without being mobilized downwards. Thereafter, the belowground biomass markedly declined due to the high respiration rate under moderate temperature. In winter, the rhizome carbohydrate stocks were consumed continuously for respiration. Finally, the rhizome biomass declined to about one-quarter of its maximum value. This value is much smaller than for *P. australis* which retains about 60% of its rhizome biomass (Schierup, 1978; Hocking, 1989; Graneli et al., 1992), or *Typha* spp., which maintains at least one-third of its rhizome biomass in late winter (Roberts and Ganf, 1986). This implies that the contribution of rhizomes as energy reserves to support new foliage development is smaller in *Z. latifolia* than in *P. australis* or *Typha* spp. In the case of *P. australis*, the TNC reserves were mobilized intensively from old rhizomes to new rhizomes before winter (Fiala, 1976; Asaeda et al., 2003; Karunaratne et al., 2004; Asaeda et al., 2006b) by terminating the oldest rhizomes which are 5 and 6 years old (Cizkova and Lukavska, 1999; Asaeda et al., 2006a). In *Z. latifolia*, such an intra-rhizome translocation mechanism is not likely because most of the buds formed in early spring emerged from the stem base or young rhizomes; the smaller size of the buds also indicates a limited amount of rhizome energy reserves to support

420 further development. Thus, the shoots have to grow by photosynthesis earlier than *P.*
421 *australis* or *Typha* spp. (Asaeda et al., 2005b).
422
423 *Nutrient dynamics*
424
425 Although the uptake of soil nutrients by roots began at a relatively early time after shoots
426 started growing, rhizome stocks were continuously used for further 2 months until May.
427 Slightly larger amounts of nutrients were consumed from rhizome stocks in 2003, as the
428 beginning of uptake was delayed until the middle of April in 2003, compared with the end
429 of March in 2002. This was likely because of the drier soil with lower nutrient
430 concentration in pore water in 2003 than in 2002. In the absence of nutrient uptake from
431 soil, shoot growth is supported by the reallocation of rhizome nutrients, which lasts until
432 the net uptake rate becomes positive (Szczepanska and Szczepanski, 1976). The nutrient
433 uptake by the roots markedly increased from June to August, associated with the vigorous
434 growth of shoots. The nutrient uptake for aboveground growth ended before September
435 with the termination of shoot growth. A significant increase in nutrients in rhizomes, i.e.
436 twice as high as before, was recorded in September or October, which was earlier than for
437 *P. australis* (Ho, 1981; Graneli, 1992). More efficient translocation of phosphorus than
438 nitrogen into rhizomes could be due to the lower ratio of N/P in pore water than the ratio
439 in plant biomass.
440 In November, when most shoots were dying, the phosphorus and nitrogen losses
441 due to shoot mortality were about 0.78 g N/m^2 day and 0.06 g P/m^2 day, respectively.
442 However, the translocation to the belowground system was only 10.1% for nitrogen and
443 18.2% for phosphorus. The small amount of nutrient translocation at the time of shoot
444 senescence was similar to *T. latifolia*; out of 0.07 g P/m^2 day loss of net living shoot loss,
445 only 0.02 g P/m^2 day was translocated downwards and 0.05 g P/m^2 day was leached out
446 or lost in dead tissues (Prentki et al., 1978).
447 The average nitrogen and phosphorus values required in the development of the
448 primary shoots were 45.4 g N/m^2 and 5.94 g P/m^2, respectively, calculated based on the *Z.*
449 *latifolia* population, while the average upward translocation of nitrogen and phosphorus
450 until May was 5.80 g N/m^2 and 0.75 g P/m^2, respectively. Therefore, only 13% of
451 nitrogen and 12% of phosphorus were covered by the reallocation from belowground
452 stocks. These values are much smaller than for other species, e.g. *T. latifolia*, which
453 translocated about 40% of phosphorus from belowground (Prentki et al., 1978; Smith et
454 al., 1988), and *P. australis*, which was reported to translocate about 55% of phosphorus in
455 Swedish productive stands (Graneli et al., 1992) and in Holland (van der Linden, 1980).

Dying shoots in spring decompose in water and sediments, supplying mineral nutrients potentially available for the community, although it takes 6 months to 1 year to decompose into mineral substances (Mason and Bryant, 1975; Vymazal, 1995; Asaeda et al., 2002; Lan et al., 2006). The total loss due to shoot mortality from May to August was 11.7 g N/m^2 and 1.6 g P/m^2 which also corresponds to approximately 40% of nitrogen and phosphorus required for aboveground. The small amount of translocation of nitrogen and phosphorus between belowground and aboveground indicates that *Z. latifolia* depends on the external nutrient cycle rather than internal translocation. This is different from *P. australis* which, even growing in nutrient-rich conditions, relies mainly on internal translocation (Kuhl and Kohl, 1993; Kuhl et al., 1997; Lippert et al., 1999). Compared with *P. australis*, the structure of *Z. latifolia* is mostly leafy and easy to collapse, and decompose in the underlying water. Therefore, the nutrient cycle is likely much faster than that of *P. australis* stands. A large proportion of roots in the belowground system, i.e. about 20%, enables fast nutrient uptake. The high mortality of small-sized spring shoots could be part of the nutrient cycle system to provide sustainable nutrients for the intensive growth of the aboveground biomass even under the low nutrient conditions, maintaining its dominance over invaders, rather than simply translocating materials inside their biomass.

Implications for management of treatment wetlands

In the management of treatment wetlands with *P. australis*, earlier harvesting of aboveground biomass is often recommended to remove nutrients from water because of the large amount of reallocation of nutrients downwards. However, this may reduce the rhizome biomass that affects the development of the foliage in the next growing season. However in case of *Z. latifolia*, nutrients for foliage development were mostly due to root uptake and the downward translocation of nutrients in autumn was very small as large percentage of nutrient was stored as in dead shoot biomass. And relatively slow decomposition rate of the dead aboveground biomass, in which the initial decomposition took about 90 days for 20% mass loss (Lan et al., 2006) is also an advantage since the harvesting time becomes more flexible. In addition, the relatively short period of heterotrophic growth combined with extensive soil nutrient uptake during the period of shoot development suggest that this plant can function as an efficient system for nutrient removal from sediment. Our findings that showed a large percentage of nutrient was stored as in dead shoot biomass are in agreement with results of few studies that demonstrated *Z. latifolia* was one of the most efficient species for nutrient removal in

constructed wetlands. For example, Iamchaturapatr et al., (2007) showed that among 18 emergent macrophytes, *Z. latifolia,* preserved the high ranking of nutrient removal rates among the plants for both area-based and weight-based calculations. Furthermore, Tanner (1996) studied about eight emergent macrophytes species and found that *Z. latifolia* was one of the species with the highest above-ground biomass values in which the plant biomass had a significant positive linear correlation with the mean removal of total nitrogen.

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641 **Figure Captions**

642

643 Figure 1. Monthly variation of (a) shoot density and shoot height, and (b) number of live
644 and dead leaves in a shoot. Values shown are mean±SD (*n*=3).

645

646 Figure 2. Daily maximum, minimum and average temperature in 2002 and 2003 (based
647 on Omiya weather station, Japan)

648

649 Figure 3. Monthly variation of dry mass of (a) aboveground and (b) belowground organs.
650 Total horizontal rhizome biomass is the sum of fresh, hard-yellow and soft-yellow
651 rhizome biomasses. Values shown are mean±SD (*n*=3).

652

653 Figure 4. (a) Daily net production and total carbon (TC) budgets of aboveground biomass
654 (AGB) and belowground biomass (BGB), (b) Daily synthesis rate of the total
655 non-structural carbohydrates (TNC) in the AGB and BGB, and (c) Daily synthesis rate of
656 the total structural carbohydrate (TSC) in the AGB and BGB. Values shown are
657 mean±SD (*n*=3).

658

659 Figure 5. Daily (a) nitrogen and (b) phosphorus net uptake, upward translocation and loss
660 due to shoot mortality. Values shown are mean±SD (*n*=3).

661

662

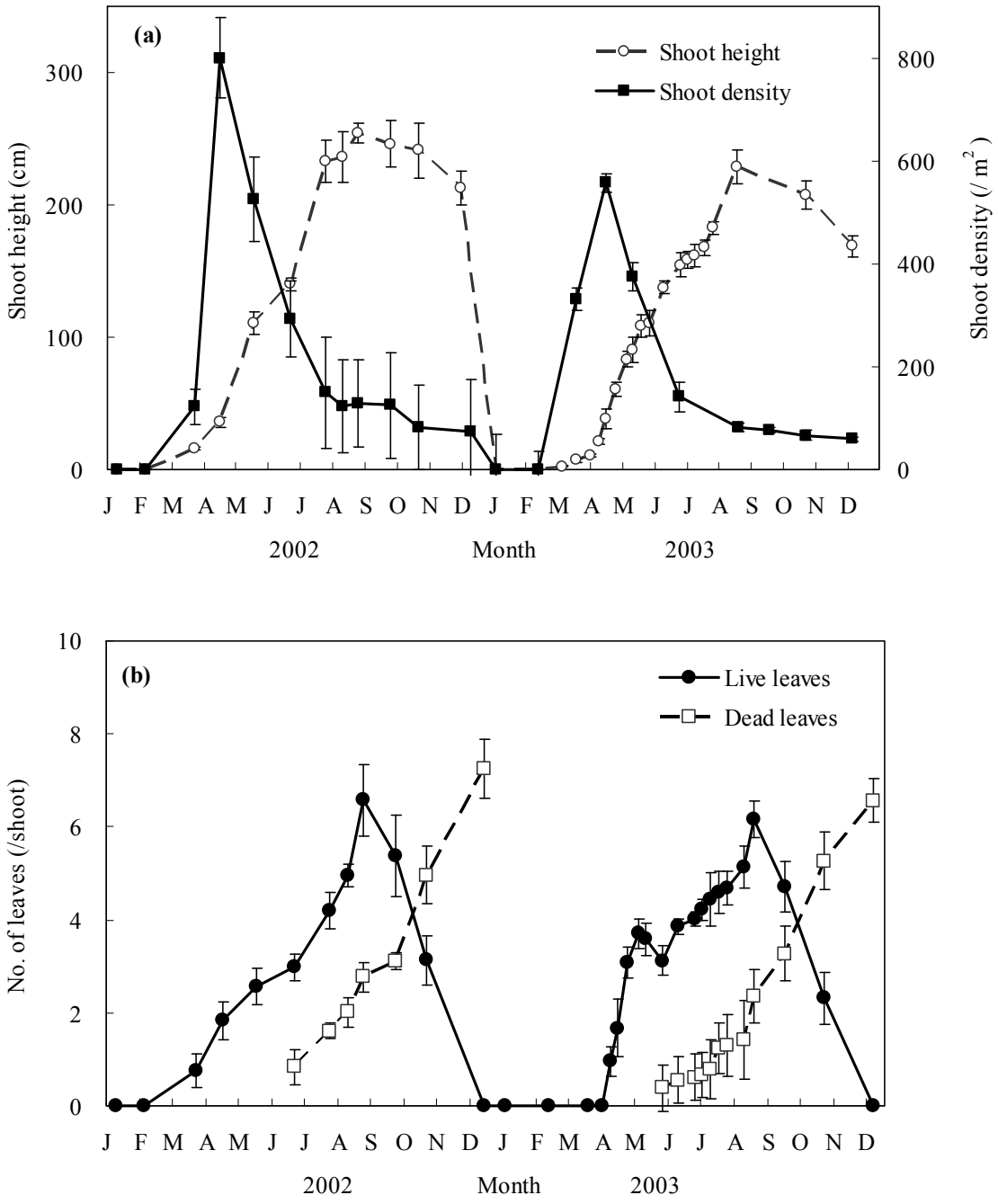
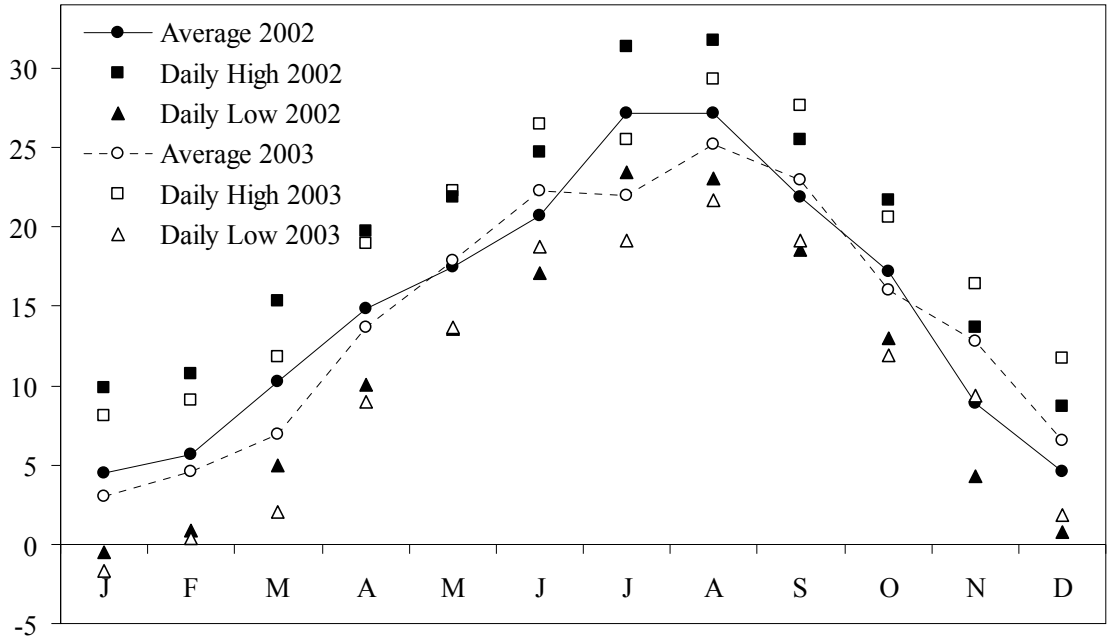


Figure 1 (a) & (b)

663



664
665 Figure. 2

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667

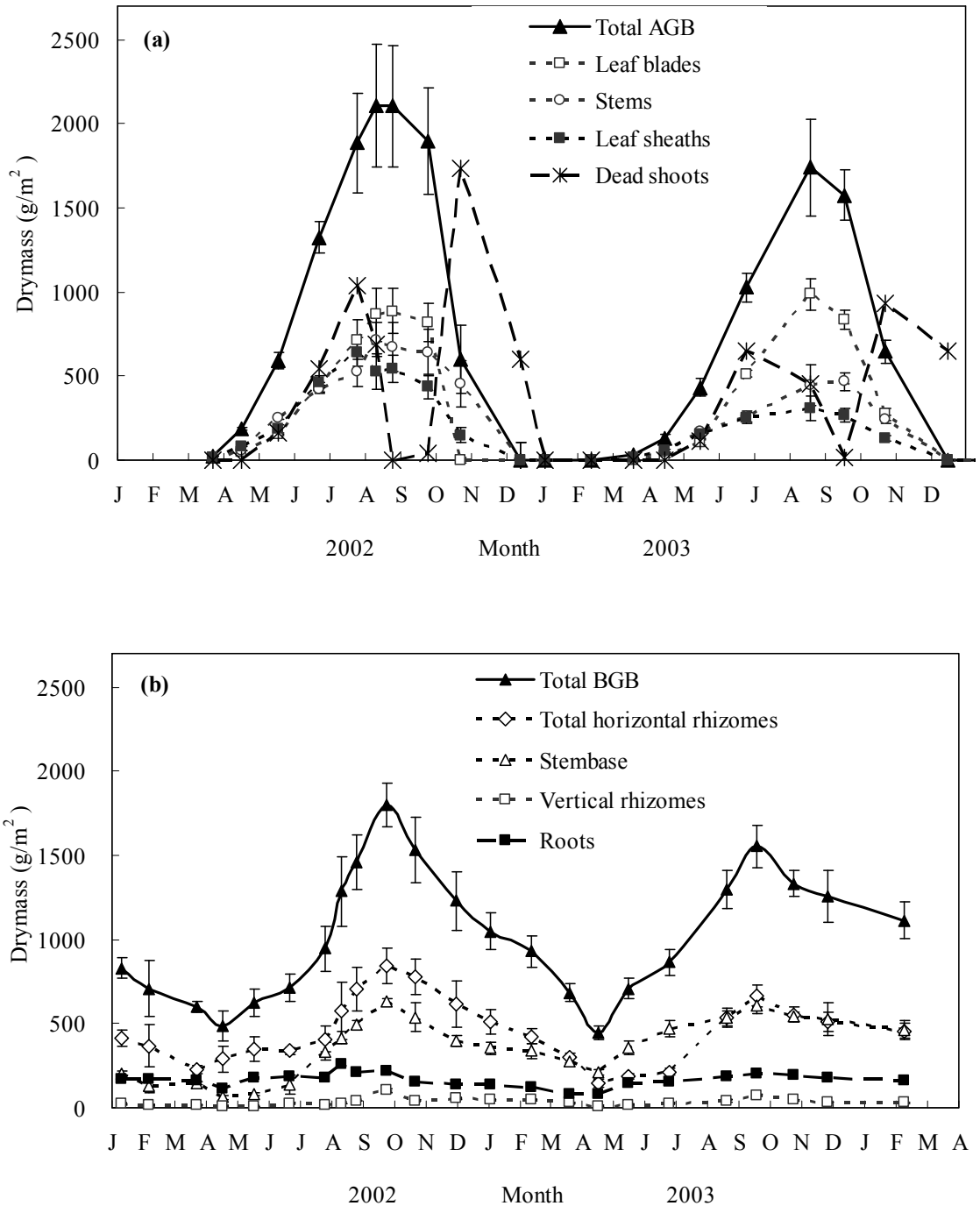
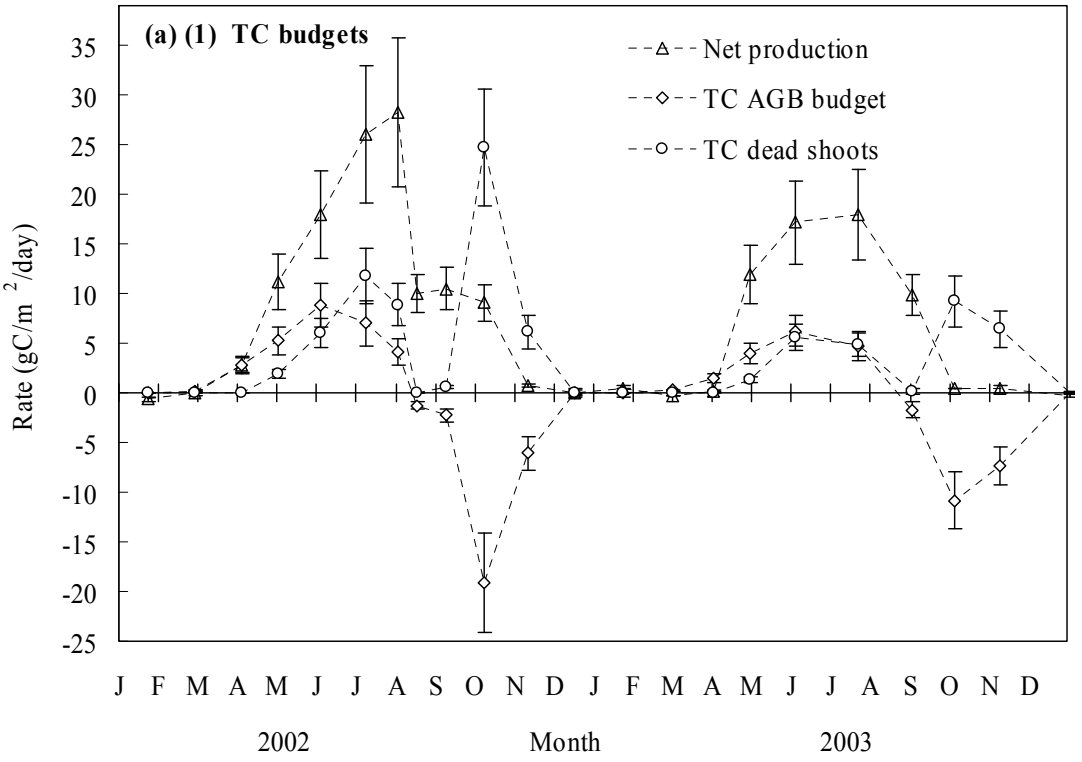


Figure 3 (a) & (b)

668



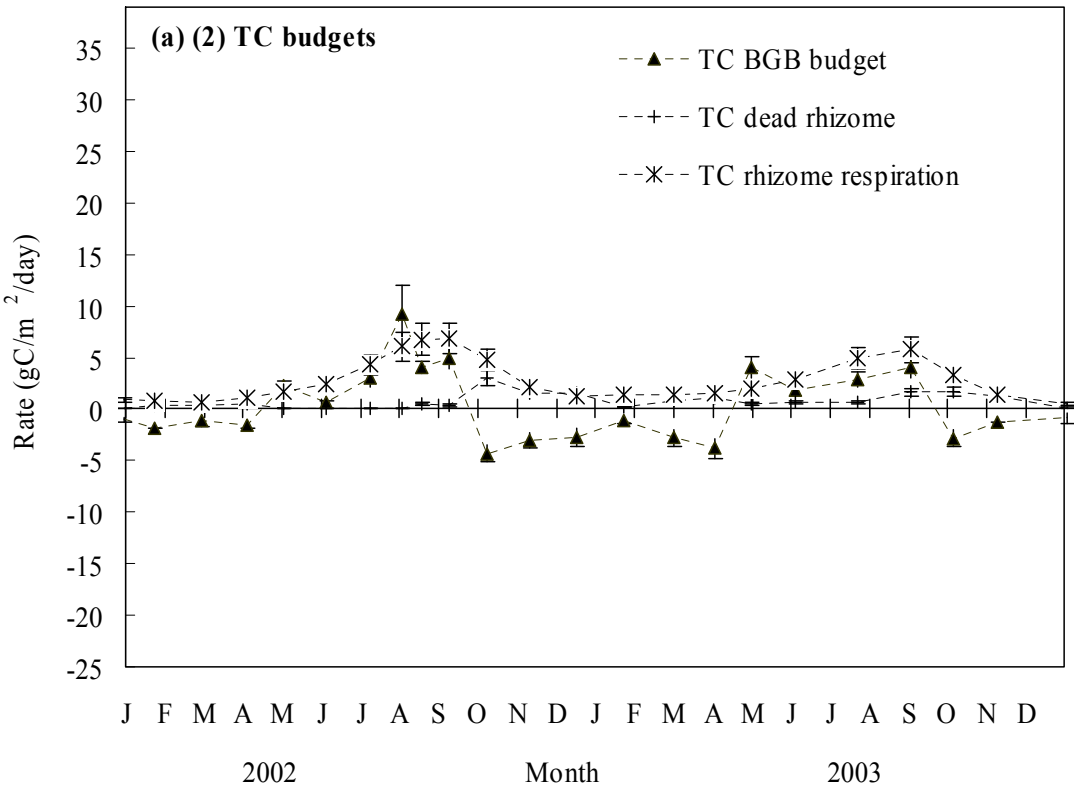


Figure 4 (a) (1) & (2)

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670

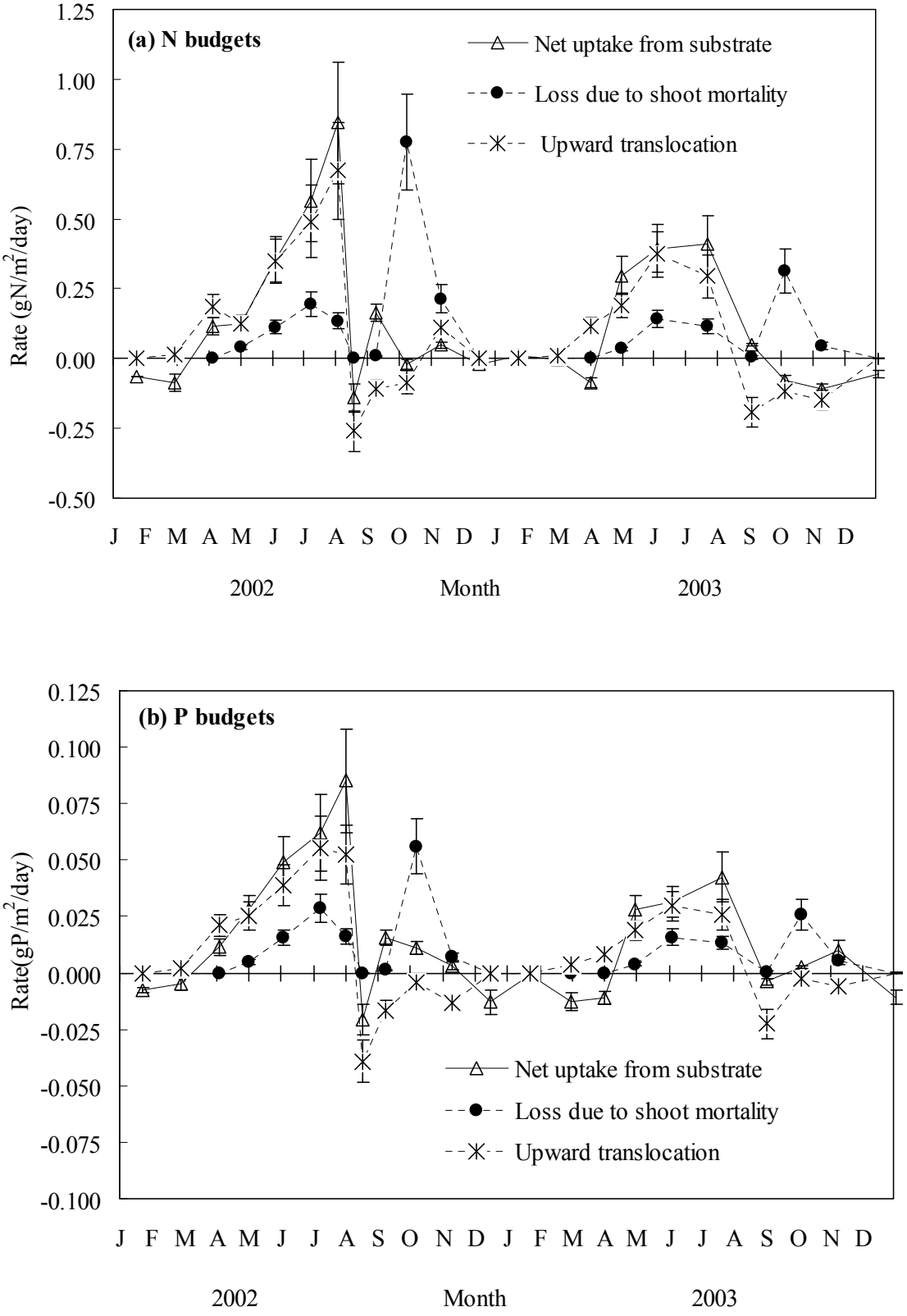

Figure 5 (a) & (b)

671