Effects of external and internal nutrient supplies on
decomposition of wild rice, *Zizania palustris*

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Abstract

How external and internal nutrient supplies affects wild rice shoot and root litter decomposition and the nitrogen (N) and phosphorus (P) dynamics in decaying litter were examined in two experiments. In a site quality experiment, wild rice shoot and root litterbags were decayed in mesocosms in the field with added N or P or a control as ambient conditions, over 115 days. In the second litter quality experiment, wild rice plants were grown with N, P, both, or none, to produce litter of different qualities and then decayed for 168 days under controlled temperature in the laboratory. The impacts of both site and litter quality was greater on shoot litter than root litter. In the site quality experiment, P addition significantly increased wild rice shoot decay more than N addition and reduced P mineralization over time compared to the control. Neither adding N nor P influenced root decay, but adding P increased root P immobilization compared to the control, and the effect was much greater in root litter compared to shoot litter. In the litter quality experiment, P enrichment increased litter quality by increasing P concentrations and water-soluble compound concentrations in shoot litter and decreasing lignin concentrations in root litter, thereby increasing decay rates of both shoot and roots, although, the impact was much greater on shoot decay. N enrichment increased plant growth but reduced shoot litter quality and decay likely because these larger plants had greater lignin concentrations for structural support and lower phosphorus concentrations and lower N:P ratios. Increased lignin likely overrides a concomitant increase in nitrogen concentration in shoot litter and appears to control wild rice decomposition. Initial lignin:P, C:P, lignin+acid-solubles:P and the N:P ratios of shoot litter were correlated to

the final mass remaining. Lignin and phosphorus appear to play a key role in driving wild rice decay through the opposite effects that each have on litter quality.

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Chapter 1. Ecological stoichiometry: physiological constraints on plant decomposition and nutrient cycling by microbes.

Energy flow through ecosystems is constrained by the proportion of nutrients in the tissue of organisms. All organisms consist of the same basic elements, of which the majority includes carbon, oxygen, hydrogen, nitrogen, phosphorus, magnesium, potassium, and sulfur. The major elements also constitute a small proportion of the earth's crust, made up of 0.18% C, 0.03% N, and 0.11% P (Lotka 1925), suggesting that living biomass is made up of nonrandom selections of elements found in the environment (Sternner and Elser 2002). Essential nutrients, such as phosphorus and nitrogen, are the most important elements required for organismal growth but supply rates are often limited by environmental factors (Elser et al. 1996). Carbon, covalently bonded to other elements in different organic compounds, provides structural support and metabolic machinery; therefore carbon makes up the majority of the mass of an organism. These relative proportions of nutrients in an organism may also be limited by genetic constraints on physiological functions such as nutrient storage capacities (Hall et al. 2005), body size (Woodward et al. 2005), and growth rates (Elser et al. 2000). In addition, genetic constraints develop through the fundamental principles of evolution and natural selection, which may directly influence stoichiometric ratios, and organisms innate ability to competition for resources and reproduction may indirectly influence such ratios (Kay et al. 2005).

Ecological stoichiometry is an effective approach to understanding how organisms respond to their environment by using the ratios of the composition of

elements in the tissue of specific organisms (Sterner and Elser 2002). The C:N ratio, or N:P ratio are useful tools for comparing the chemical makeup of organisms. Groups of organisms have characteristic C:N and N:P ratios that define their chemical makeup and nutrient requirements. Similarities are found between the major kingdoms and phyla; animals have C:N ratios around 4-17, plants have greater ranges of around 20 to 100+, bacteria 10, and fungi 12. Species functions, habitat, and mobility also have a strong influence on nutrient and carbon requirements. Animals require more nitrogen for building muscles (Elser et al. 1996), and vertebrates need greater phosphorus for skeletal support, while immobile plants require greater carbon for structural support (Sterner and Elser 2002). In addition, the N:P ratio is more fixed in some organism while others show greater plasticity. For example, although plankton exhibit some plasticity in their N:P ratio (≤ 7.2), the range of their N:P ratio is not as great as the external supply, suggesting there are some limitations to the range of N:P ratios, such as physiological storage capacity for nutrients (Hall et al. 2005). Other organisms, such as bacteria and fungi, exhibit a rather fixed N:P ratio of around 6.9, even across the globe (Cleveland and Liptzin 2007).

Differences between adjacent trophic levels in these ratios can have a great impact on the energy flow through food webs. If a consumer has a different ratio than their food, the consumer must retain, excrete, or take up additional nutrients in order to convert their food into new biomass. Therefore, the ratios between trophic levels can be used to predict what limits and drives the energy and nutrients through food webs, communities and entire ecosystems (Sterner and Elser 2002). Any changes in one trophic level in a food

web may have cascading effects. For example, Elser et al. (2000) demonstrated how manipulating the top predator of a lake food web altered not just the structure of the food web but changed the external N:P supply in the water column. This occurred because the addition of a top-predator to a lake changed the composition of species, and subsequently these species had different tissue N:P ratios that eventually lead to an increase in the dissolved N and P supply in the lake. This study demonstrates the impact that differences in stoichiometric ratios among species can have on the nutrient cycle of an entire ecosystem.

The greatest difference in C:N ratios between adjacent trophic levels occurs between plants and their consumers, including both herbivores, which consume green plants, and microbes, which consume dead plant material. These large differences may have great implications for the flow of carbon and nutrients in ecosystems. In fact, converting plant material of the relatively wide C:elemental ratios, that are characteristic of plants, into consumer or microbial biomass of much narrower ratios is often the rate limiting step in the flow of carbon and nutrients in ecosystems and leads to the excretion of excess nutrients in their food by consumers and uptake of nutrients of insufficient supply in litter by microbes. Microbes, particularly, bacteria and fungi, make up the greatest number of organisms in terrestrial systems and are the main organisms that break down plant litter during decomposition. These microbes consume or digest plant organic matter and convert the litter into proteins, carbohydrates, lipids, nucleic acids and energy during decomposition and then release nutrients back into the sediments after decomposition. The turnover of nutrients during decomposition is also a major source of

nutrients for both microbes and plants and the main source of energy for microbes (Berg and McClaugherty 2008). In addition, decomposition can occur over many months, years, and sometimes decades depending on the plant material (Parton 2007). Thus, the duration of decomposition can limit plant growth insofar as it determines the turnover time of nutrients.

Consequently, the large disparities between the C:N and sometimes C:P ratios between plants and microbes often require that microbes retain or take up additional amounts of N and/or P as they convert plant litter into their biomass (Paul 2007). Nitrogen is one of the most important essential nutrients for both plants and microbes. Plants more often take up the inorganic forms of nitrogen as ammonium and nitrate, but most nitrogen in soil is in the organic form, unavailable for most plants (Brady 1974). This leads to competition for the inorganic form of nitrogen between microbes and plants when both are nitrogen limited (Melillo et al. 1984, Brady 1974). Microbes, however, often out-compete plants for nitrogen (Kaye and Hart 1997) because their surface area in the soil is orders of magnitude greater than that of plant roots. In addition, microbes often need more nitrogen in proportion to their biomass because of their narrower C:N ratio. Microbes have a C:N ratio of approximately 10, much narrower than dead plant material with a C:N ratio of at least 25 and often greater. Therefore, during decomposition, plant litter can only supply 1 unit of nitrogen for every 25 units of carbon, but microbes need 1 unit of nitrogen for every 10 units of carbon in order to produce 1 unit of biomass. This causes microbes to *immobilize*, or take up and retain inorganic nitrogen from the surrounding sediment, during decomposition as they distill off carbon in respiration. The

nitrogen immobilized as organic nitrogen in microbial biomass is unavailable for plant uptake until it is released (Paul 2007). As microbes exhaust the more easily decomposable carbon compounds in the plant litter, such as sugars and starches, the population growth slows and eventually net immobilization ends. The nitrogen immobilized in microbial biomass is then released during *mineralization* when the microbial biomass decays and the nitrogen is released during lysing or is released as metabolic waste in the inorganic form, usually ammonium and nitrate (Brady 1974). The timing of return of nutrients released back into the sediment is therefore controlled by the disparity between microbial and plant litter C:N ratios and thereby influences the growing conditions of the plants the following year. In addition, difference in storage capacities of nutrients leads to different turnover times depending on the nutrient. Nitrogen is often stored in the organic form in plants (Twilley et al. 1986) and for this reason nitrogen is often not as readily leached during the initial phase of decomposition, which further limits nitrogen availability until microbial digestion. In contrast, plants have varying proportions of inorganic soluble phosphorus to organic phosphorus, which can influence the amount of phosphorus leached (Twilley et al. 1986).

Plant litter quality influences the timing of nutrient turnover during decomposition (Berg and McClaugherty 2008). The C:N ratio in plants varies greatly between species, habitat, growth form and plant part. Plants with high litter quality tend to have greater nutrient concentrations and less fiber content, thus lower C:N or C:P ratios, allowing for more rapid digestion by microbes (Chimney and Pietro 2006, Godshalk and Wetzel 1978). Plant litter with high C:N ratios >25 leads to microbial immobilization because

there is not enough nitrogen present in plant tissue for microbial use (Keeney and Sahrawat 1986, Kaye and Hart 1997), resulting in reduced decomposition rates (Berg and McClaugherty 2008). Often the rate of nitrogen mineralization increases with decreasing C:N ratios (Enquezor 1976). Phosphorus immobilization can also occur during decomposition of plant litter (Puriveth 1980, Paul 2007, Morris and Lajtha 1986), especially when the substrate for microbial growth has a C:P > 300 P immobilization, and C:P ratios less than 200 generally leads to net mineralization (Paul 2007).

Other environmental factors can influence plant decomposition including temperature, moisture, oxygen, and pH levels (Berg and McClaugherty 2008), although new evidence suggests these may not play as great of a role as the litter quality. A recent 10-year decomposition study examined terrestrial decay in seven biomes across the globe and found nitrogen concentrations of initial leaf litter and mass remaining of all but one study site was more important in predicting nitrogen mineralization than climate, or soil nutrients and microbial communities (Parton et al. 2007); N mineralization began once litter C:N was approximately less than 40, suggesting the strong limitation that plant tissue chemistry has on the timing of nutrient cycling and decomposition. However, Rejmankova and Houdkova (2006) found site quality to have a greater impact on aquatic plant decay, though litter quality was also significant.

Some ecosystems, specifically aquatic systems, may be especially constrained by the disparity of the C:N ratio between plants and microbes. Nutrient availability and microbial activity may play a greater role in decomposition when temperature and water are not limiting (Vitousek 2004), as is the case in some aquatic systems. Detritus is the

primary source of carbon and energy in wetlands (Polunin 1984). While there are several types of aquatic plants found in wetlands, emergent macrophytes often contribute a substantial amount to primary production (Wetzel and Howe 1999). In addition, a relatively low proportion of aboveground biomass is removed through consumption by herbivores, besides some occasional cases of high grazing; therefore, most of organic matter in aquatic systems is reduced through decomposition by the microbes (Polunin 1984). Thus, nutrient cycling and energy flow through many wetlands may be primarily through decomposition of emergent plants (Corstanje et al. 2006).

Wild rice (*Zizania palustris*) is a useful model plant for research on how differences in stoichiometry of plant litter and microbes influence not only nutrient cycling but also population dynamics. Natural stands of wild rice undergo population cycles of approximately 3-5 years (Archibold et al. 1989) and recent evidence suggests these cycles are likely due to a delay in available nitrogen because of microbial immobilization during decay of the wild rice litter (Walker et al. 2010). Wild rice often grows in monotypic stands with few other aquatic species. Therefore, examining this species alone may shed more light on the role of microbial immobilization of N in relation to productivity and population dynamics compared to mixed vegetative communities with greater diversity of species and litter chemistries. Nutrient cycling in wild rice and how microbial processes control it was addressed in this study by integrating across several fields including ecological stoichiometry, ecology, plant physiology and biogeochemistry. The emphasis of this experiment was on the effects of both the internal and external nutrient supplies on wild rice decomposition and included

two separate experiments. In one experiment, nitrogen and phosphorus were added to sediment with decaying wild rice litter to determine if differences in the external nutrient supply affect wild rice litter decay. The second experiment examined how difference in litter quality affects wild rice decomposition by first growing wild rice plants with different N and P supplies to change litter qualities and then decaying this litter.

Furthermore, research on nutrient cycling in aquatic systems is needed to improve our understanding of fundamental principles in wetland ecology. In addition, there is and has been substantial loading of nutrients into aquatic systems from anthropogenic inputs including runoff from agriculture production, mining, and fossil fuel production which could have great implications for wetlands and nutrient cycling (Rabailas 2002).

Wetlands have been found to retain the greatest amount of nitrogen in comparison to other water bodies, suggesting the importance of these systems in predicting effects of nitrogen loading into water bodies and in the nutrient cycles (Saunders and Kalff 2001). Also, land-use changes from dams, construction, and logging can also influence decomposition and nutrient cycling (Webster and Benfield 1986). Research on the effects of external nutrient input into aquatic systems will help further improve our abilities to reduce such impacts and preserve existing pristine wetlands. Finally, *constructed wetlands* have been successfully designed for mitigation or treatment of human wastewater (Axler et al. 2001) and additional research in this field would further help improve this technology. Understanding how and what types of wetland plants retain, filter, and release nutrients would help designers develop created wetlands with greater nutrient retention and slower mineralization rates.

Chapter 2. Effects of external and internal nutrient supplies on decomposition of wild rice, *Zizania palustris*

INTRODUCTION

Natural stands of wild rice (*Zizania palustris*) cycle every three to five years but the causes of the cycle are not completely understood (Archibold *et al.* 1989, Pastor and Walker 2006). Kennan and Lee (1988) found wild rice productivity declined 5 years after seeding in a controlled field experiment, and the decline was highly correlated to a decrease in nitrogen availability in the sediments. Recent evidence suggests that wild rice might self-induce cycles because of the asynchrony in the timing of nitrogen uptake and release of nitrogen during litter decay (Pastor and Walker 2006, Walker et al. 2010). More than half of nutrient uptake of wild rice occurs within a short 10-15 day period early each summer, which is followed by a period of rapid growth (Grava and Raisanen 1978). While most nutrients are released during early stages of wild rice litter decay, the majority of nitrogen remains tied up in the litter and is not released until later in the growing season or even after the growing season of the following year (Sain 1984, Walker et al. 2010). Therefore, the retention of nitrogen in litter beyond the critical growth period of wild rice leads to a delay in the supply of nitrogen relative to plant uptake. This delay in nitrogen available to growing plants in one year is due to microbial immobilization of nitrogen during the decay of litter from the previous season and over time such delays likely lead to populations cycles (Walker et al. 2010). Immobilization occurs because the disparity between the narrow C:N ratio of microbes (7-12) versus the

wider C:N ratio of plants (>25) causes microbes to take up additional nitrogen from the sediments in order to digest the carbon in plant tissue for energy and growth during decomposition, which reduces available nitrogen to plants (Paul 2007). Understanding the influence of the nitrogen cycle on wild rice production, especially with respect to litter decay, may help further explain the mechanism behind wild rice population cycles.

Phosphorus is another essential element that can limit plant growth, although phosphorus does not appear to be as limiting to wild rice growth as nitrogen (Walker et al. 2010). Typically, phosphorus in plant litter is lost rapidly through an initial leaching phase then continues to decrease at a slower rate throughout the rest of the decomposition (Thormann et al. 2001), while usually very little nitrogen is lost through the leaching phase (Park and Cho 2003). Leaching occurs initially when dissolved water-soluble compounds are released rapidly after the litter contacts water or sediments and is not the same as microbial digestion of plant matter during decay (Polunin 1984). Phosphorus leaching rates are likely related to the proportion of soluble inorganic phosphorus in plant material and not initial total phosphorus concentrations (Twilley et al. 1986). Microbial immobilization of phosphorus, like nitrogen, can also occur during decay of some plant litters (Puriveth 1980, Paul 2007, Morris and Lajtha 1986), especially when the substrate for microbial growth has a C:P > 300 ; a C:P < 200 generally leads to net mineralization (Paul 2007). Morris and Lajtha (1986) found that nitrogen was immobilized during decay of *Zizania aquatica* leaves, and not phosphorus, but nearly half of the original phosphorus content still remained by the growing season 2 years later because release

was slow. A slow release in phosphorus availability may also occur in related species and cause a delay in the P supply for *Zizania palustris*.

Decomposition of plants is influenced by internal tissue chemistry, or litter quality, which varies greatly between species (Webster and Benfield 1986). Generally, high quality litter decays faster because of high nutrient concentrations, narrow C:N and C:P ratios, and low cellulose or lignin concentrations (Chimney and Pietro 2006, Godshalk and Wetzel 1978, Melillo et al. 1984). Plant litter with wider C:N ratios (>25) leads to microbial immobilization (Keeney and Sahrawat 1986, Paul 2007). While the C:N ratio can often predict decay rates in many terrestrial (Taylor et al. 1989, Cheshire and Chapman 1996) and aquatic plants (Chimney and Pietro 2006, Twilley et al. 1986), the lignin:N (Xie et al. 2004, Melillo 1984) or the concentration of total recalcitrant compounds (Godshalk and Wetzel 1978) may be a better index for litter quality and decay. The C:N ratio may not be the best predictor of decay because plant litter is composed of different types of carbon compounds that behave differently during decomposition. Sugars and starches are the primary substrate for microbes in plant litter and are decayed faster (Berg and McClaugherty 2008), while recalcitrant compounds, like lignin and cellulose, are more difficult to digest and lead to slower decay rates (Melillo *et al.* 1984, Devevre and Horwath 2000). In addition, while there are several types of aquatic plants, emergent macrophytes can contribute a substantial amount to primary productivity in wetlands (Wetzel and Howe 1999) and generally decay slower (Twilley et al. 1986) because of the greater fiber needed for structural support above water (Polunin 1984, Webster and Benfield 1986). Therefore, litter quality and

decomposition of emergent plants may be particularly important in determining energy flow and nutrient cycling in aquatic systems (Corstanje et al. 2006).

The effect of external nutrient supplies from the environment on plant decomposition is not as well understood as the internal effects of litter quality (Berg and McClaugherty 2008). In aquatic systems, changes in the external supply of nutrients can have either no effect on plant decomposition (Chimney and Pietro 2006, Newman et al. 2001), reduce decay rates (Thormann et al. 2001), or increase decay rates (Villar et al. 2001, Corstanje et al. 2006, Lee and Bukaveckas 2002, Debusk and Reddy 2005, Thormann et al. 2001). Immobilization of N and/or P sometimes occurs with nutrient addition depending on species and the nutrient (Puriveth 1980, Paul 2007, Chimney and Pietro 2006, Morris and Lajtha 1986, Debusk and Reddy 2005). If nutrient addition increases decay and immobilization, this may be a sign of nutrient deficiencies of the substrate for microbes, which could correspond to increased microbial activity (Corstanje et al. 2006) or increased microbial biomass (Qualls and Richardson 2000). There are also mixed results on whether litter quality or external nutrient supply has a greater effect on plant decomposition, although studies have found both to be significant (Corstanje et al. 2001, Lee and Bukaveckas 2002, Rajmankova and Houdkova 2006).

Many studies on plant decomposition do not simultaneously consider the influence of both shoots and roots on nutrient cycling (except see Lan et al. 2006, Wrubleski et al. 1997, Thormann et al. 2001, Walker et al. 2010). Often roots decay slower than stems and leaves (Webster and Benfield 1986, Brock et al. 1985). Wild rice roots decay slower than shoots due to the greater recalcitrant compounds in roots, and

over time, the net amount of nitrogen immobilized is greater in root litter (Walker et al. 2010). How the external environment influences the quality of root versus shoot litter or the decay of each tissue is not well understood, especially in regards to recalcitrant compounds.

This study examined the influence of both external and internal supplies of N and P on wild rice litter decomposition in order to further investigate the mechanism behind the delay in the nitrogen supply, which is thought to cause population cycles. Changes in the nutrient supply to growing plants may affect plant root and shoot chemistry, which consequently may influence decay rates and the timing and release of nutrients. Nutrient dynamics could determine the next year's plants litter chemistry. In addition, external supply of nutrients in the sediment may also influence decomposition rates, which Rejmankova and Houdkova (2006) describe as "site quality".

The main goal of this study was to determine the effects of both the internal and external nutrient supplies on wild rice root and shoot decomposition and nutrient dynamics in decaying litter through two experiments. Experiment 1, *Site quality*, examined the influence of *external* nutrients on decomposition rates by adding N and P to sediments with decomposing wild rice litter produced from unfertilized plants. Experiment 2, *Litter quality*, examined the influence of *internal* plant tissue content on the subsequent root and shoot decomposition by adding N and P during wild rice plant growth and then comparing the resulting root and shoot litter tissue chemistry, including nutrient, total carbon and lignin concentrations and subsequent decay rates.

METHODS

Effects of nutrient addition on decay (Site Quality Study)

The first experiment was conducted at the University of Minnesota Duluth Research and Field Studies Station in Duluth, Minnesota. Wild rice litterbags were placed in tanks with wild rice plants growing in an experiment of nitrogen or phosphorus addition to investigate how nutrient availability, or *Site Quality*, affects the decay of wild rice litter of the same chemistry. Plants were grown in polyethylene tanks (132 cm long x 79 cm wide x 63 cm deep) buried to ground level with overflow drainpipes connected to separate polyethylene overflow buckets. Plants were grown in the same conditions as described by Walker et al. (2006) with the only modifications being all tanks were thinned to 30 plants and fertilizer was applied as described below. Eighteen tanks were randomly assigned to N, P, or a control treatment (ambient conditions), yielding 6 replicate tanks per treatment. Water temperature averaged 19°C over the summer and pH averaged 7.87 in control tanks, 7.78 for tanks with added N, and 8.07 for tanks with added P.

Litterbags contained wild rice litter from unfertilized plants grown in a shallow pond outside the Swenson Science Building, at the University of Minnesota Duluth. Senesced plants were sampled in 2007. Roots were picked clean of sediments and the litter was then stored in a cold room at 4°C until used in this study. Shoot and root litter separated, cut into 2-4 cm pieces, and each thoroughly mixed. Approximately 3 g of litter was weighed and sealed in a 1 mm mesh plastic-coated litterbag. A total of 54 litterbags were assembled for each tissue type and placed in tanks on June 2, 2009. Three litterbags

per tissue type were placed in each of the 18 tanks (6 litterbags per tank). Root litterbags were placed into the sediment just below the surface and shoot litter was placed on the top of the sediment and held in place with small PVC sticks. Previous research demonstrated that placement in or on top of sediment had a negligible effect on decay compared with differences in litter chemistry between roots and shoots (Walker et al. 2010). Three replicates of litterbags used in a previous litter decomposition experiment (Walker et al. 2010) were sufficient to statistically detect differences in decay and immobilization rates.

Application of fertilizer began approximately one week after germination, June 11, 2009, prior to floating leaf stage and continued weekly through September 3, after seed maturation and followed the phenology of N and P uptake in wild rice plants according to Grava and Raisanen (1978). The amount applied was lowest earlier and later in the growing seasons and greatest during emergent growth (July 9 – 23). Nitrogen as NH_4Cl and P as mono-basic $\text{Na}_2\text{PO}_4 \cdot 7\text{H}_2\text{O}$ fertilizer was first diluted with 135 to 270 mL of water, thoroughly mixed, then inserted with syringes through 1-cm diameter perforated PVC tubes that were submerged into the sediment of the treatment tanks. Total amount applied was determined based on individual plant requirements of approximately 0.3 g N per plant (1.15 g NH_4Cl) and 0.11 g P per plant (0.95 g $\text{Na}_2\text{PO}_4 \cdot 7\text{H}_2\text{O}$).

Three litterbags were sampled out of a randomly assigned tank on the following days after placement in tanks: 9, 14, 21, 35, 65, and 105. Litter used in this experiment is noted as $+P_S$ for litter decaying in tanks with P added to the sediments and $+N_S$ for litter

decaying in tanks with N added to the sediments. Litterbags were prepared for tissue chemistry analysis as described below.

Effect of nutrient enrichment on litter quality and on decay (Litter Quality Study)

For the second experiment, wild rice was grown the field also at the University of Minnesota Duluth Research and Field Studies Station in Duluth, Minnesota with the addition of nutrients during growth one season to determine the effects of these nutrients on the quality of litter produced and its subsequent decay. Wild rice seeds were collected from wild rice plants growing in the same pond (described earlier in site quality study) at the University of Minnesota, Duluth. Wild rice sediments were taken from a natural wild rice bed in the Fond du Lac Band of Lake Superior Ojibwe Reservation, Carlton County, MN. Seeds were planted in 12 separate 77-liter buckets (53.3 cm diameter x 43.2 cm height) and seedlings thinned to a density of 12 plants per bucket (60 plants/m²). Four cm of sand was first placed in each bucket followed by 10 cm of sediments. Sediments were analyzed using the CHNS Analyzer, available P Bray's Extraction (Bray and Kurtz 1945), and organic P using digestion and had 10.41 % C (+/- 0.23), 0.980 %N (+/-0.016), 0.058 % organic P (+/-0.003), and 0.42 ug/g available P (+/-0.162). Water was added from a well on site to each bucket to a depth of approximately 36 cm and maintained at this depth throughout the growing season. Well water was sampled and analyzed for chemical content; NH₃-N and NH₄-N concentrations were always less than from 0.2 ug ml⁻¹. Rain levels were collected using a rain gauge on site and chemistry was monitored; NH₃-N and NH₄-N ranged from 0.2 to 1.99 ug ml⁻¹. The buckets with the plants and

sediments were buried in the ground up to a depth of 36 cm to maintain water temperatures.

Plants were grown in a factorial experiment of 2 nitrogen levels crossed with 2 phosphorus levels (high vs. control) to investigate: 1) how nutrient availability influences litter chemistry of roots and shoots and 2) how litter chemistry of these two tissues affects decay and nitrogen and phosphorus dynamics. Four buckets per treatment were randomly assigned to each combination of N and P additions to provide enough litter material for 6 sampling periods for roots and 7 for shoots during the subsequent litter decay experiment to produce 72 roots litterbags (3 replicates) and 112 total shoot (4 replicates). Application of fertilizer was applied using the same methods described above for the site quality experiment (the total amount was less because fewer plants were grown).

Entire plants with senesced leaves, stems and roots were harvested after the last seeds had fallen at the beginning of October. Roots were picked clean of sediments and washed in deionized water. Plants were dried at 60° C for five days. All shoot litter, including stems and leaves were combined from the four buckets (replicates) within each treatment, chopped into 1-2 cm lengths, thoroughly mixed, then separated into 1-2 g sub-samples to create four replicate litterbags per treatment sampling date. All root material was combined and processed the same as shoots, then separated into 0.7 – 1 g sub-samples to produce three replicate litterbags per each treatment sampling date. Litterbags were created using nylon stockings with ≤ 1 mm mesh size. Litterbags were placed in Mason jars with the excess untreated sediment from the field experiment to provide a microbial inoculum. Jars were flooded to 2 cm above the litterbag surface then incubated

at 19 °C for 6 months in the dark as described in Vandermeulen (2002); the only modification was that the temperature was set to 19 °C to simulate natural mid-summer temperatures measured previously at the same study site (Dewey and Pastor, unpublished data). Four shoot and three root litterbags were destructively sampled at 2, 4, 6, 8 (shoots only sampled at 8 weeks), 12, 16, and 24 weeks. Litter produced for this experiment will be noted as the following: N_{Enriched} as N *enriched* litter; P_{Enriched} for phosphorus *enriched* litter, or litter produced from plants grown with adding P to the sediments; and NP_{Enriched} as N and P *enriched* litter produced from plants grown with adding both N and P.

Chemical analyses of litter samples

All litterbag samples were first rinsed with deionized water then dried at 60°C for five days and weighed to determine mass loss. Dried litter of each individual litterbag was ground through a cyclone sample mill with 30 mm screen or a IKAWerke MF 10 mill with a 0.25 screen. Total plant tissue C, N, and P were determined on a sub-sample from each litterbag. Approximately 0.9 g of plant litter sub-samples were digested with 5.0 mL H₂SO₄ and 5 mL H₂O₂ then analyzed for P by a molybdate-antimony-ascorbic acid method on a Lachat Autoanalyzer (Lachat Instruments method 13-115-01-1-B). Total C and N were determined on 3-7 mg sub samples of each litterbag with a Thermo Scientific FLASH EATM 1112 Elemental Analyzer. Acid-insoluble (lignin and recalcitrant carbon compounds), acid-soluble (cellulose, hemicellulose), water-soluble (simple sugars and polyphenols) and nonpolar extractables (fats, oils, and waxes) were analyzed on the initial and final litter samples in the site quality experiment and all litter bags in the litter

quality experiment using modified techniques described by Ryan *et al.* (1990). All values of lignin, acid-soluble, water-soluble and nonpolar extractables are ash-free.

Data analysis

Several models were used to investigate the rate of litter decay, rate and duration of immobilization, time of mineralization, and amount of nitrogen and phosphorous immobilized for both experiments. The rate of litter decay was modeled using a double exponential decay equation because of the different rates of decay for water soluble, acid-soluble and acid insoluble extractables (Minderman 1968):

$$M(t) = M(0)s_1e^{-k_1t} + M(0)s_2e^{-k_2t} \quad (1)$$

where M is the litter biomass at time t , s_1 is the labile fraction decaying at a rate k_1 and $s_2 = 1 - s_1$ is the recalcitrant fraction decaying at a rate k_2 . Approximate parameter estimations were determined by nonlinear regression with SigmaStat 3.5 (Systat Software Inc. 2006), which applied the Marquardt-Levenberg algorithm to solve for the parameter coefficients (the sums of squared residuals of Eq. 1 were minimized to a tolerance of 0.0001). Starting values were chosen based on results from nonlinear regressions of the same double exponential model for a similar wild rice litter decomposition study (Walker *et al.* 2010). The double exponential model would collapse to a single exponential model if the best estimate of $s = 1$. Nonlinear regressions were run on mass remaining and time as days 7 through day 115 for the site quality study or day 7 through 168 for the litter quality study.

The fraction of the original mass of N, or P, remaining at time t (also referred to as *N or P content*) was calculated by first taking the N/P concentrations at time t , in

values from 0 to 1, multiplied by the fraction of original mass remaining at time t to determine the N/P fractions of original mass, then dividing by the initial N or P concentrations at time 0:

$$\frac{\%N_t, \%P_t \times \text{fraction OM remaining}_t}{\%N_0, \%P_0} \quad (2)$$

N or P content was plotted against time in days to determine the timing of immobilization and mineralization. A net immobilization of N or P occurs when this fraction increases and net mineralization of N, P occurs when this fraction decreases.

Aber and Melillo (1982) and Melillo et al. (1984) have shown that net amount of N immobilized during decay can be predicted from the parameters of a regression of % organic matter (%OM), or original mass remaining, at time t vs. the nitrogen concentration at time t :

$$\%Original\ mass_t = a - b (\%N_t) \quad (3)$$

where % organic matter remaining and %N values ranged from 0 to 1. The slope, b , and intercept, a , of this linear function were applied in a separate function to estimate the net amount of exogenous nitrogen immobilized ($N_{\text{immobilized}}$):

$$N_{\text{immobilized}} = \left[\left(\frac{a \times 100^2}{4b \times 100} \right) - N_0 \right] \times 0.1 \quad (4)$$

where N_0 equals the initial nitrogen concentration of the litter and $N_{\text{Immobilized}}$ represents the mg of N immobilized per g initial litter. Eq. 4 was modified from the original only by applying the units of concentrations from 0 to 100 rather than 0 to 1. Inspection of the data indicated an initial period of rapid nitrogen and phosphorus loss occurred over approximately 7 to 9 days before immobilization began (Fig 1). While this rapid loss may

not be primarily due to leaching, it will still be referred to as a “leaching” phase. To account for this loss in decaying root and shoot litter, day 9 (site quality) or day 7 (litter quality) was set to be day 0 of the immobilization phase and the original mass remaining in Eq. 4 was recalculated. Though this experiment was not designed to test effects of physical leaching (without microbial inoculum), an estimate of the amount of nutrients lost during this period was still of interest because prior research did not show a rapid loss of N in wild rice (Walker *et al.* 2010). Therefore, the total amount N and P in grams released during the leaching period were calculated by taking the difference of the concentration of N/P multiplied by the total weight in grams of litter at day 0 and day 9 (site quality) or day 7 (litter quality).

One-way factorial ANOVA's were run using SigmaStat 3.5 (Systat Software Inc. 2006) to compare differences in the initial tissue chemistries, amount of N or P leached, and the fraction of original mass remaining on the last sampling day between control and each treatment (N alone, P alone, and, in the litter quality experiment, N and P combined). Two-way factorial ANOVA with an interaction term was not used to test for treatment effects because after running both types of tests, it was determined that the One-way ANOVA more accurately detected significant differences between the N, P, and NP enrichment treatments, and the control litters, while Two-way ANOVA sometimes reported significant interactions between N and P when there was no significant difference between the NP treatment and the controls because of opposite effects of N enrichment treatment and the P enrichment treatment. Significance differences were tested with Tukey and Hold-Sidak *t*-tests; those reported are between the treatment

described and the control only. Initial tissue chemistry values were transformed using an arc sin of the square root transformation or natural log transformation when appropriate to meet assumptions of normality or equal variances; transformed results rarely improved significant results of original data. Chemistry values reported are the averages of the original values, not back transformed. Repeated measures (RM) were run to compare the effects N or P addition, or enrichment, on the fraction of original mass remaining over time and on the N and P content over time. For the litter quality study only, average initial chemistry values per treatment were linearly regressed against fraction mass remaining at the final sampling date, both decay rates (k_i), the proportion of labile (s_1) or recalcitrant pools (s_2), or the maximum amount of N and P immobilized to determine the best initial litter quality predictors for litter root decay.

RESULTS

Effects of nutrient addition on decay (Site Quality Study)

Shoot and root litter used in the Site Quality Experiment was from one pool of unfertilized litter and, therefore, all litterbags in this experiment had the same initial tissue chemistries prior to each nutrient addition treatment. The only difference between the chemistries of the tissues was shoot litter had significantly greater water-soluble compounds soluble (simple sugars and polyphenols) and significantly less acid-soluble compounds (cellulose, hemicellulose) than roots (Table 1). No significant differences were found between root and shoot litter N, P, or lignin concentrations, or the N:P, C:N, C:P, lignin:N, or lignin:P ratio.

In the field, shoot litter decayed faster than root litter across both nutrient additions (Fig. 2, Table 2). Loss of the original mass remaining in shoot litter over time was best fit by the double exponential decay model (Eq. 1), while root litter was best fit using a single exponential decay model (first part of Eq. 1 with one litter fraction ($s = 1$) and one decay constant (k)) (Table 2). The double exponential model accounted for 98% or more of the variations in mass lost from all shoot litters. The single exponential model accounted for 94% of the variation in mass lost from control root litter, 98% of mass loss of +P_S root litter, and 86% of the variation of mass loss of +N_S root litter.

Shoot litter decay was initially dominated by rapid decay of the labile pool followed by a period dominated by slower decay of the recalcitrant pool (Table 2, Fig. 2). In contrast, root litter had a single pool that decayed slower than shoots. N addition had no significant effect on shoot or root decay over time ($F_N = 1.046$ $p = 0.364$, roots $F_N = 1.982$, $p = 0.232$). While N addition increased shoot litter decay rates (k_i), the greater proportion of the recalcitrant pool overrode the faster decay rates, perhaps explaining the lack of a significant effect over time, however, final mass remaining on last day was significantly lower (37.4%, $p = 0.020$). P addition significantly increased shoot litter decay over time (shoot $F_P = 22.145$ $p = 0.009$) but had no effect on root decay over time (root $F_P = 0.165$ $p = 0.705$). Adding P reduced both decay rates (k_i) in shoot litter but the proportion of labile compounds greatly increased, overriding the slower decay rates (Table 2); final mass remaining on last day was significantly reduced (16.6% $p < 0.001$). N addition slightly reduced the root litter decay rate, while P addition had no effect on

root litter (k , Table 2). N or P addition did not significantly affect final mass remaining in root litter on last day (56.7% to 66.2% original P remaining, all $p > 0.05$, Fig.A-1).

The addition of either N or P had no significant effect on N content over time in either tissue (shoot $F_N = 3.863$, $p = 0.121$, shoot $F_P = 0.080$, $p = 0.791$; root $F_N = 2.609$, $p = 0.182$, root $F_P = 1.197$, $p = 0.336$) and did not significantly affect N leaching in either tissue (Table 2, Fig. 4). However, shoot litter leached greater N than root litter under all treatments and had overall greater mineralization of N throughout the decay period (Fig. 2, 4). Initially shoot litter rapidly lost over half of the original N by the first 9 days (Fig. 4), while N content in root litters had a much slower N loss by day 9 and approximately 89% to 96% of original N remained (Fig.A-1).

After the initial leaching phase, root litter immobilized greater N (8.8 to 9.3 mg N/g initial mass) and over a longer period of time than shoot litter (5.9 to 6.1 mg N/g initial mass) (Table 2, Fig 3). Shoot litter had a small peak in N immobilization at day 9 and possibly later in decay, except that the timing of immobilization in $+N_S$ litter was not apparent. N addition increased N immobilized in root litter but not shoot litter. P addition had no effect on the net amount of N immobilized in root litter but possibly increased the amount of N immobilized in shoot litter (Table 2), although, comparison to control shoot litter was not possible because net N immobilized could not be calculated due to an insignificant correlation in Eq 3 ($r^2 = 0.0$, $p = 0.998$, Table A-1).

By the final sampling day, shoot litter lost greater N (34 to 43% N remained) than root litter (82 to 104% N remained) (Fig. 3) and neither N nor P addition significantly affected either (all $p > 0.05$, Fig.A-1).

N addition had no significant effect on the P content over time in either tissue (Fig 3, RM shoot $F_N = 0.201$ $p = 0.677$; root $F_N = 0.286$, $p = 0.621$). P addition significantly increased the P content over time in both tissues (RM shoot $F_P = 32.489$ $p = 0.004$; root $F_P = 107.409$, $p = 0.001$). N addition had no effect on the amount of P leached in either tissue (Fig. 4). By day 9, all shoot litter had released over half of the original P, while root litter had 87% to 94% of original P, except +PS root litter had 128% (Fig.3) because P addition significantly inhibited P leaching in root litter and instead P was immobilized (Table 2, Fig. 4).

After the initial leaching phase, shoot litter mineralized greater P (-0.07 to -0.12 g P/g initial mass) and more rapidly than root litter (-0.03 to -0.04 g P/g initial mass) (Table 2); most of this occurred prior to day 65, after which little change occurred in P content (Fig.3). In root litter, a small peak in immobilization occurred after day 9, although over time the net amount of P was still mineralized. N addition did not affect P immobilization or mineralization in either tissue (Fig 3, Table 2). P addition lead to a small amount of P immobilized in shoot litter and likely a great amount in root litter, although the amount could not be predicted due to an insignificant correlation in Eq. 4 ($r^2 = 0.10$, $p = 0.206$, Table A-1).

By the last day, shoot litter had released greater P (7% to 8% of original P remained) than root litter (41 to 45% of original P remained, Fig 3), except P addition significantly increased final P content in both shoot litter (23% P remained, $p < 0.001$) and root litter (70% P remained, $p < 0.05$, Fig A-1) compared with controls.

Effect of nutrient enrichment on litter quality (Litter Quality Study)

N and P enrichment affected plant root and shoot initial litter quality. Initial total C was not affected by enrichment and ranged from 41.9% to 46.2% in both root and shoot litter (Table 1). Initial N concentrations ranged from 0.68 to 1.19% in shoot and 0.72 to 1.03% in root litter and were significantly greater in control root litter than control shoot litter. The only effects from either enrichment were N enrichment significantly increased N concentrations in shoot litter and P enrichment significantly increased N concentrations in root litter.

Initial P concentrations ranged from 0.03 to 0.16% in shoot litter and 0.10 to 0.33% in root litter with no significance difference between control shoot and root litters (Table 1). The only effect from enrichment was P enrichment significantly increased P concentrations in shoot litter, while N enrichment significantly reduced P concentrations in root litter.

Initial C:N ratios of all litter ranged from 38.3 to 67.4; control shoot litter was not significantly different than control root litter (Table 1). Initial N:P ratios ranged from 5.1 to 38.0 in shoot litter and 4.3 to 19.9 in root litter, control root litter had a significantly wider N:P ratio than control shoot litter. The only effect of enrichment on either tissue was N enrichment significantly narrowed the C:N ratio in shoot litter and widened the N:P ratio in both tissues. P enrichment significantly narrowed the C:N ratio in root litter. NP enrichment significantly widened the C:N ratio of root litter.

Total carbon was further analyzed for labile and recalcitrant compounds (Table 1). Initial lignin concentrations were 1.6 to 7.5% in shoots and 4.5 to 9.3% in roots; water

soluble concentrations were 18.1 to 28.7% in shoot litter and 10.1 to 16.1% in root litter; and acid-soluble concentrations were 60.1 to 67.0% in shoot litter and 69.8 to 80.5% in root litter. Control roots had significantly greater lignin concentrations, lignin:N ratio, lignin:P ratio and significantly less water-soluble concentrations than control shoots. The only significant effect of either enrichment on these concentrations or ratios was: N enrichment increased lignin and the lignin:P ratio in shoot litter ; P enrichment increased water-solubles and decreased the lignin:P ratio in shoot litter and decreased lignin and the lignin:N ratio in root litter; NP enrichment increased lignin, the lignin:N, and lignin:P ratio in shoot litter.

Effect of litter quality on decay (Litter Quality Study)

Shoot and root litter followed similar decay patterns: an initial rapid decay of the labile pool was followed by an extended period of slower decay of the recalcitrant pool. Shoot litter always decayed faster than root litter (Fig 2, Table 2). Shoot litter had on average 19.8% to 42.1% of original mass remaining after 168 days, compared to roots that had on average 44.3% to 55.6% of original mass remaining. The decay of all root and shoot litter was best fit by the double exponential model (Eq. 1). This model accounted for 93% of the variation in mass loss for N_{Enriched} root litter and more than 95% of the variation in mass loss for P_{Enriched} root litter, NP_{Enriched} root litter, and control root litter and all shoot litter (Table 2, Fig 2).

N enrichment significantly slowed shoot litter decomposition over time ($F_N = 295.345$ $p < .0001$, Fig 2), while P enrichment significantly increased shoot litter decomposition over time ($F_P = 448.284$ $p < .0001$). There was also a significant

interaction of N and P ($F_{NP} = 45.539$ $p < .0001$); NP enriched litter decayed more rapidly during the initial period perhaps likely due to the effect of P and the greater labile pool, but then decay slowed more than control litter likely due to effect of N. N and NP enrichment reduced both decay rates (k_i). Final mass remaining on last sampling day was significantly greater in N_{Enriched} litters (42.1%, $p < 0.001$) and NP (29.3%, $p = 0.001$) relative to controls, but the mass remaining of P_{Enriched} litter (19.8%) did not differ from that of control litter ($p = 0.275$); therefore the impact of P enrichment was greatest during the initial decay period because P_{Enriched} shoot litter had a slightly greater labile pool.

N enrichment had no effect on root litter decomposition over time ($F_N = 1.614$ $p = 0.0002$, Fig. 2), while P enrichment significantly increased root litter decomposition over time ($F_P = 43.305$ $p = 0.0002$), and the effect of both N and P did not have a significant interaction ($F_P = 511$ $p = 0.495$). N_{Enriched} and NP_{Enriched} root litter had slower decay of the recalcitrant pools. Final mass remaining of root litter at the last sampling date was not significantly affected by enrichments (44.3% to 55.6%, all $p > 0.05$). Therefore, the impact of P enrichment on root decay was likely during the initial decay period, similar to shoots. P_{Enriched} roots had the smallest labile pool but the fastest decay, which compensated for the greatest recalcitrant pool with a decay rate similar to control litter.

N enrichment had no effect on shoot litter N content over time ($F_N = 0.464$ $p = 0.509$), but significantly reduced N content in root litter over time ($F_N = 6.911$ $p = 0.030$, Fig.3). P enrichment significantly reduced N content over time in both shoot litter ($F_P = 26.224$ $p < 0.001$) and root litter ($F_P = 8.506$ $p = 0.019$). However, P enrichment reduced

N content earlier in shoot litters, while P enrichment reduced N content over the entire time relative to the control litter.

Initially, shoot litters lost N more rapidly than root litters. Shoot litters lost over half of the original N by day 7 and then a more gradual loss (if any) occurred, and N_{Enriched} and P_{Enriched} shoot litters had significantly greater N leached (Fig. 3, Table 2). In root litters, N was released more slowly; approximately 84% to 93% N remained by day 9, except in P_{Enriched} and NP_{Enriched} root, which litter leached a significantly greater amount of N (Fig 4) and around 73% of P remained in each (Fig 3).

After the initial leaching phase, both tissues immobilized N during various times during decomposition, however, greater N was immobilized in root litter (7.8 to 8.9 mg N/g initial mass) and over a longer time than in shoot litter (5.0 to 7.0 mg N /g initial mass, Table 2). N_{Enriched} shoot litter immobilized more N, which may have occurred briefly after day 9. P_{Enriched} shoot litter had a slightly greater amount of N immobilized to control, which likely occurred after day 84 (Fig 3). In root litters, N_{Enriched} litters had slightly less (if any) amount of N immobilized which may have occurred after day 84. P_{Enriched} root litters had slightly more N immobilized, which began after day 9 and continued through the last day.

By day 168, shoot litter had less N content (34% to 45% remained) than root litter (72 to 99% remained) and enrichment did not affect shoot or root litters final mass remaining when compared to control litters (all $p > 0.05$, Fig A-1).

Generally all litters had greater P loss than N, regardless of tissue or treatment (Fig 3). N enrichment significantly increased P content over time in both shoot litter (F_N

= 65.734 $p < 0.0001$) and in root litter ($F_N = 72.034$ $p < 0.0001$). In contrast, P enrichment significantly decreased P content over time in both shoot litter ($F_P = 64.651$ $p < 0.0001$) and in root litter ($F_P = 9.326$ $p = 0.016$).

An initial leaching phase likely occurred within the first 7 days and was much greater in shoot litters than root litters. Shoot litters released the most P during the first 7 days when around 17 % to 27% of original P remained, except N_{Enriched} shoot litter had approximately 50% of original P remaining and leached a significantly smaller amount of P (Table 2, Fig 3, Fig 4). Root litter released P more slowly; around 80% to 96% of original P remained by day 7, except P_{Enriched} root litter had 55% of original P remaining (Fig 3) and a significantly greater amount of P leached (Table 2, Fig 4). N enrichment did not affect P leaching in root litter (Table 2, Fig 4).

Root litter immobilized a small net amount of P (0.47 to 1.02 mg P/g initial mass) more than shoot litter (0.16 to 0.45 mg P/g initial mass), except for P_{Enriched} root litter that mineralized a small amount of P (-0.11 mg P/g initial mass) (Table 2). N and NP enrichment reduced the amount of P immobilized in shoot litter, while P enrichment had no effect, but the timing of immobilization was not apparent in shoot litters and likely occurred throughout decomposition. Root litter immobilization likely occurred after day 85, except in P_{enriched} root litter than exhibited a small peak around day 14 (Fig 3). N enrichment reduced the amount of P immobilized in root litter while NP increased it.

By day 168, nearly all shoot litter had considerable less P content (17% to 24% of original P remaining) than root litter (68% to 89% P remaining), except for N_{Enriched} shoot litter that had significantly greater P remaining than control shoots (38%, $p >$

0.001) and P_{Enriched} root litter had significantly less P remaining than control roots (25%, $p < 0.05$)(Fig A-1).

Lignin concentrations generally increased in both shoot and root litter throughout decomposition (Fig A-2). Lignin concentrations were greatest in NP_{Enriched} and N_{Enriched} shoot litter throughout decomposition, more than both control and P_{Enriched} litter. In root litter, lignin concentrations were similar and generally increased through decomposition, except that lignin concentrations in N_{Enriched} root litter increased rapidly during the first few weeks then after a brief decline, increased similarly to other treatments.

Predictors of the final mass remaining on the last sampling day of initial shoot litter variables included in order of decreasing significance: lignin:P, C:P, lignin+acid-soluble:P and the N:P ratio (all $r^2 > 0.90$ $p < 0.05$, Fig. 5, Table A-2). Acid-soluble concentration in shoot litter was also correlated to the proportion of the labile pool (s_1 ; $p = 0.022$) and recalcitrant litter pool (s_2 ; $p = 0.032$), and lignin concentration in shoot litter was correlated to recalcitrant decay rates (k_2 ; $p = 0.007$). The only significant predictor of final mass remaining in root litter on the last day was the initial concentration of water-soluble compounds ($p = 0.048$;). Initial root litter lignin concentration was also correlated to the labile decay rate (k_1 ; $p = 0.043$).

DISCUSSION

Effects of nutrient addition on decay (Site Quality Study)

P addition to sediments increased wild rice shoot litter decay the greatest and reduced P mineralization compared to ambient conditions. In contrast, N addition to sediments only increased decay of shoot litter by the last day of the study. Roots decayed much slower than shoots, likely due to greater acid-soluble compounds (lignin) and less water-soluble compounds. Walker et al. (2010) also reported wild rice root litter decayed slower than shoots, while others have supported this in some but not all emergent graminoid species (Puriveth 1980, Brock et al. 1985). Neither N nor P addition affected root decay but P addition significantly increased P immobilization in roots. Overall, the combined results suggest that high P loading in one year would likely stimulate shoot litter decay, which may reduce P availability to growing plants because of increased immobilization by microbes in both shoot and root litter. This provides new evidence that the site quality, has a greater impact on wild rice shoot decay compared to root decay and P limitation by microbes is likely playing a key role in wild rice decay and nutrient mineralization.

While nutrient addition did not affect leaching (except when P was added to root litter), there was still a substantial amount of both N and P leached in wild rice shoot litter. Shoot litters also leached a slightly greater fraction of P than N, which Twilley et al. (1986) suggests is because decaying plant litter tends to release greater P than N initially because of the proportion of water-soluble P fractions and because plants may store N in the organic form. In contrast to our results, other studies have reported N

immobilization initially in wild rice decay but not N leaching (Walker et al. 2010, Morris and Lathja 1986). Although, Sain (1984) found a very low amount of N was released initially and much more P was released in decaying wild rice litter, which was more similar to our results. Differences in nutrient leaching in studies on wild rice appear to differ with species, site conditions and sampling protocol. In addition, nutrients leaching from wild rice litter may not be available to growing plants and may be lost to the water column, although, this may be more likely to occur in lakes or riparian systems with greater flowing water. Nutrients leached and mineralized from wild rice roots throughout decay may provide more nutrients than shoot litter because of their location in sediments where nutrients may not be as readily flushed away.

The effect of site quality on aquatic plant decomposition does not have consistent results in the literature, even for decaying graminoids. N and P addition did not affect the decay of *Schoenoplectus californicus* (Ca. bulrush) and *Cyperus giganteus* (giant flatsedge) (Villar *et al.* 2001). Corstanje et al. (2006) found N and P addition increased leaf decay of *Typha* (cattail) but not *Cladium* (saw grass) and increased microbial activity and P immobilization in both litters but did not change N content, suggesting P limitations in microbes. Qualls and Richardson (2000) reported that P addition stimulated decay of the same two species, increased microbial biomass and increased P content but did not alter N content, which they suggest was because the greater N:P ratio in litter provided enough N but not enough P for microbes, that were likely P limited. These two studies support and help explain our results. The N:P ratio of wild rice shoot and root litter in the present site quality study (6.9 and 6.6, respectively) was very close to the

microbial average (6.9, Cleveland and Liptzin 2007). Cleveland and Liptzin (2007) provide evidence that measuring the microbial tissue N:P ratio, but not plant litter or sediments, may be a more accurate method to determine nutrient limitations of ecosystems because microbes are driving decomposition. Therefore, wild rice litter in the site quality experiment may have been on the lower end of the average, explaining the stimulatory effect of added P to decaying shoot litter. Also, the C:P ratio in both shoot and root litter was greater than 300, which is the limit where P immobilization is likely to occur (Paul 2007). Perhaps adding P allowed microbes to use the excess carbon as a substrate for growth and decay shoot litter more rapidly than ambient conditions. The null effect of added P on root decay concurrently with increase in P immobilization in root litters suggests microbes were likely stimulated and growing, but the microbes were likely unable to digest root litter faster because the lower water soluble concentrations and greater acid soluble in root litters.

Site quality affects litter quality & litter quality affects decomposition

N enrichment reduced shoot litter quality and decomposition, while P enrichment increased shoot litter quality and stimulated decay. While N enrichment enhanced plant growth, as expected, since N limits wild rice plant growth but P does not (Sims et al. *submitted*, Walker et al. 2010). Enhanced growth required greater structural support that was provided by significantly greater lignin concentrations, which likely reduced decay. However, N_{Enriched} shoot litter had a C:N ratio of 38, the lowest of all litters, while the control shoot litter was significantly wider (67), suggesting the importance to distinguish between total carbon verses recalcitrant carbons in wild rice tissue and decay. Thus, the

C:N ratio may not be a suitable index for litter quality, particularly when comparing litters with greater C:N ratios (>25). N enrichment also significantly slowed P mineralization over time but did not affect N content over time, suggesting that microbes digesting N_{Enriched} plant shoot litter either required greater P than N because of P limitations, or there was less organic P to mineralize. The N:P ratio in N enriched plants was greater (> 19 in both tissues) than the average microbial N:P ratio (6.9, Cleveland and Liptzin 2007), therefore less P was available in plant tissue relative to microbial needs. This lends additional support to the site quality study that microbes may be P limited in wild rice beds. In addition, N enrichment in similar emergent graminoid species may also create further consequences on litter qualities and decay. Emergent macrophytes often contribute substantial amounts to primary production in wetlands (Wetzel and Howe 1999). Consequently, such effects from nutrient loading in the environment may be particularly important in understanding nutrient cycling in aquatic systems.

In contrast, P enrichment did not enhance wild rice growth but increased shoot and root litter quality and decay, although the impact was less on roots. P_{Enriched} shoots initially increased leaching amounts of N and P and significantly increased both N and P mineralization over time in both shoots and roots. Wild rice plants take up added P through luxury consumption but this enrichment does not increase plant biomass (Walker et al. 2010, Sims et al. submitted). Consequently, P_{Enriched} shoots had significantly greater concentration of water-soluble compounds and the lowest lignin:N and N:P ratios, and root litter had greater P and N concentrations, low lignin concentrations, and the lowest

N:P and C:P ratios, both suggesting P enrichment produces higher quality litter. Water-soluble compounds are more easily digested by microbes, leading to faster decay, and greater leaching rates (Berg and McClaugherty 2008), which may explain the greater leaching of N and P in P_{Enriched} shoots. If P enrichment increases decay and greater P and N are leached and mineralized into the sediments, particularly in root litter, this may increase available N and P for the following year's plants. In contrast, if N enrichment slows shoot litter decay and reduces P mineralization in both shoot and root litter, less P may be available for the following year's plants. As discussed earlier, nutrient mineralization may be more important in root decay than shoots because of loss of nutrients through the water column and retention in sediments.

Rejmankova and Houdkova (2006) found P enriched litters decayed faster, had greater leaching of P and also had greater microbial biomass. They believe this was due to a positive feedback created through P enriched litters: P stimulates microbes that break down plant material, which further releases P and stimulates microbes again. Their study lends additional support to our results and helps explain the stimulatory effect of P. Twilley et al. (1986) found initial P concentrations were not good indicators of the leaching rates and suggested that rather greater P leaching is related to the proportion of soluble inorganic phosphorus to organic phosphorus in plant litters.

Modeling Plant decomposition

The double exponential model should be considered when modeling aquatic plant decay over an extended period of time. The double exponential model explained shoot and root litter decay well. The approximated values for both decay rates and the proportion of the

two litter pools were similar to those reported in Walker *et al.* (2010). Many decomposition models for aquatic plants use a single-pool first-order model (Chimney and Pietro 2006); although decomposition models including labile and recalcitrant compounds have been successfully used for forest (Minderman 1982), grassland (Hunt 1977) and aquatic species (Brock *et al.* 1985). This model accurately estimated slower decay rates for roots and the recalcitrant litter pool, which has been seen in other aquatic plants (Brock *et al.* 1985) including wild rice (Walker *et al.* 2010).

Comparison to other studies was difficult since few studies have used a double exponential model for aquatic grasses (except Walker *et al.* 2010). Therefore, data from each experiment was fit to single exponential models for comparison purposes only. Decay rates from both experiments ranged from 0.0042 to 0.019 day⁻¹ for shoots and 0.0029 to 0.005 day⁻¹ for roots, which are comparable to other aquatic emergent macrophytes. A review by Chimney and Pietro (2006) calculated a mean decay rate of 0.0330 day⁻¹ from three studies on *Zizania aquatica*, a related wild rice species, which is greater than our values but possibly due to difference in species and the site conditions. In addition, the mean decay rates for 22 terrestrial and aquatic graminoid species from the review ranged from 0.0005 to 0.0520 day⁻¹. The range of decay values from our study are approximately 28% of their range, indicating the full impact that both litter quality and site quality can have on the decomposition of a single species.

Litter quality predicts decay of wild rice

Initial chemistry parameters of shoot litter that were good predictors of final mass remaining included P or lignin (lignin:P, C:P, lignin+acid-soluble:P, N:P ratio), except

that acid soluble concentrations in shoot litter predicted the proportion of labile and recalcitrant pools (Fig. 4, Table A-2). Therefore, wild rice shoot litter quality parameters including P or lignin may predict decay rates and may be more useful without N. The limited predictors for root parameters support the lack of an effect that litter quality has on root decay. Only initial water-soluble compounds significantly correlated with final mass of roots remaining suggesting this may be the only parameter in root litter that is affected by external nutrient supplies. Except for N:P and C:P ratios, the best predictors of both shoot and root decay did not include nitrogen and carbon, suggesting that while N influences growth, it may not best represent litter quality and decay. In addition, the C:N ratio, a common predictor of plant decay was not correlated to any decay parameters.

Because the effect of enhanced lignin production on N_{Enriched} shoots overrode the effect of greater N concentrations, lignin was a more suitable predictor. Some authors found the lignin:N ratio or total recalcitrant compound concentration, instead of the C:N, to be better predictors of plant decay in aquatic systems (Melillo et al. 1982, Xie 2004, Godshalk and Wetzel 1978b). In this study, lignin:N ratio was not a good predictor ($p = 0.3378$ not shown) of shoot decay, although the lignin:P was the best predictor of shoot decay. This supports our hypothesis that microbes in wild rice beds are likely P limited and are stimulated when P loading occurs to the sediments.

As discussed earlier, the N:P ratio of the microbial biomass may be a better indicator of ecosystem limitations than plants litter quality (Cleveland and Liptsin 2007) and may help explain how decomposition will proceed in certain conditions. Therefore,

measuring initial plant litter will help predict decay but the site conditions must also be considered.

Whether site or litter quality has a greater impact on plant decay obviously depends on species, litter quality, growth form and plant part. Rejmankova and Houdkova (2006) found site quality to have a greater impact on aquatic plant decay than litter quality, but Corstanje et al. (2006) found the litter quality effect was greater than the site quality. The present study suggests that both litter quality and site quality have a significant impact on aquatic plant decomposition (Table 3), whether one is greater or lesser in a given year may not be as important as the greater impact on nutrient cycling for the following year's plants.

Wild rice root litter immobilized greater N and over a longer time than shoot litter, both in Walker et al. (2010) and this study, and was likely due to the greater concentration of recalcitrant carbon compounds in roots (Walker et al. 2010). Our study provides additional evidence for the hypothesis of Walker et al. (2010) that roots may be a major cause of a delay in available N for the following year's plants because of their slow decay and high N immobilization. This study also suggests that shoot decay may play a role, especially when lignin concentrations are increased to support larger plants during years of high production, leading to slower decay and less P mineralization. Nutrients released from shoot litter depend on both litter quality and site quality. Therefore, leaching and mineralization of N and P from shoots may be delayed or sped up depending on the quality of litter produced the previous year and the external supply of nutrients during the current year.

Table 1. Initial tissue chemistry values of wild rice, *Zizania palustris*, senesced shoot or root litter prior to decomposition studies. Site quality litter was from unfertilized plants; Litter quality litter was from plants enriched with either phosphorus (P), nitrogen (N) or both (NP) and controls plants were grown with ambient conditions. Values are means of three to four replicate litter bags with standard error bars in parenthesis. Significant difference to control litter represented by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Study	Tissue	Treat	%C	%N	%P	C:N	N:P	C:P	%NPE	%WS	%AS	%Lignin	Lig:N	Lig:P
Site Quality	Shoot	none	45.6 (0.2)	0.854 (0.0545)	0.124 (0.0095)	53.9 (3.8)	6.9 (0.2)	370.7 (28.4)	3.70 (0.94)	18.4 (0.43)	70.9 (1.13)	7.03 (0.42)	8.35 (1.0)	57.4 (6.7)
			44.9 (0.3)	0.857 (0.003)	0.132 (0.009)	52.3 (0.3)	6.6 (0.43)	342.9 (22.5)	6.61 (1.11)	7.99*** (0.36)	76.8** (0.23)	8.62 (1.04)	10.06 (1.19)	64.9 (4.3)
		Control	45.3 (4.3)	0.675 (0.0453)	0.105 (0.0073)	67.4 (5.8)	6.5 (0.7)	437.7 (47.0)	10.7 (2.0)	19.8 (1.9)	66.8 (2.21)	2.67 (0.31)	4.049 (0.612)	25.5 (2.621)
Litter Quality	Shoot	P	41.9 (0.1)	0.824 (0.0531)	0.164* (0.0175)	51.5 (3.7)	5.1 (0.5)	263.2 (25.3)	5.73 (1.16)	28.7* (2.2)	63.9 (3.37)	1.64 (0.15)	2.46 (0.603)	12.1** (2.303)
		N	44.6 (0.2)	1.193** (0.106)	0.031** (0.0013)	38.3** (3.5)	38.0*** (2.4)	1431.1*** (66.5)	7.34 (0.79)	18.1 (1.6)	66.99 (0.76)	7.54*** (0.59)	6.59 (1.02)	243.6*** (26.434)
		NP	44.2 (0.1)	0.785 (0.0244)	0.086 (0.010)	56.5 (1.8)	9.5 (1.2)	537.6 (63.3)	5.85 (0.28)	27.4 (1.9)	60.1 (1.73)	6.62*** (0.646)	8.42** (0.713)	78.8*** (7.36)
Root	Control	Control	46.2 (0.3)	0.872** (0.0196)	0.102 (0.0061)	53.1 (1.35)	8.6* (0.4)	456.5* (27.6)	6.5 (2.0)	11.1** (0.42)	73.1 (2.21)	9.31*** (0.40)	10.7*** (0.494)	92.2*** (7.4)
			44.7 (1.0)	1.029* (0.047)	0.328** (0.0727)	43.7* (1.8)	4.3* (1.8)	186.9** (76.7)	10.6 (2.4)	15.13 (2.69)	69.8 (2.55)	4.45* (1.59)	4.21* (1.36)	25.0 (17.5)
		P	44.1 (0.5)	0.907 (0.0307)	0.046 (0.0012)	48.8 (2.1)	19.9* (0.50)	972.5*** (38.1)	2.61 (0.24)	10.15 (0.57)	80.45 (1.67)	6.79 (1.26)	7.64 (1.661)	151.4 (32.46)
	NP	NP	44.6 (0.5)	0.715* (0.027)	0.096 (0.0057)	62.6* (2.8)	7.51 (0.34)	469.6 (24.6)	3.09 (0.75)	16.07 (0.87)	71.8 (1.01)	9.03 (0.62)	12.73 (1.19)	94.8 (6.7)

Table 2. Decay parameters approximated from a nonlinear regression of the double exponential model of wild rice, *Zizania palustris*, decaying over 115 days in the field (site quality study) or 168 days in the laboratory (litter quality study). Predicted net amount of N and P immobilized over the course of the study and total amount N and P leached within first 7 days (site quality) or 9 days (litter quality) of the studies is also shown.

	Treat	% OM final	s ₁	s ₂	k ₁	k ₂	r ²	N immob (g/g M ₀)	P immob (g/g M ₀)	N leach (g)	P leach (g)
SITE QUALITY											
SHOOTS	Control	49.6 (2.7)	0.32	0.68	0.075	0.0031	0.98	n/a	-0.12	0.010 (0.001)	0.00189 (0.00023)
	P	16.6 (1.5)	0.86	0.15	0.025	0.0018	0.99	6.13	0.64	0.00884 (0.0009)	0.00165 (0.00012)
	N	37.4 (1.9)	0.21	0.80	0.120	0.0066	0.98	5.88	-0.07	0.00776 (0.0018)	0.00180 (0.00002)
ROOTS	Control	60.8 (0.9)	0.98	-	0.0047	-	0.94	8.75	-0.03	0.00097 (0.0005)	0.00012 (0.00002)
	P	56.7 (2.3)	0.99	-	0.0050	-	0.98	8.76	n/a	0.00042 (0.0004)	-0.00049* (0.00004)
	N	66.2 (1.2)	0.98	-	0.0042	-	0.86	9.26	-0.04	0.00123 (0.0004)	0.00023 (0.00016)
LITTER QUALITY											
SHOOTS	Control	22.4 (0.80)	0.223	0.777	0.253	0.0079	0.98	5.29	0.40	0.00684 (0.00034)	0.00203 (0.00005)
	P	19.8 (0.82)	0.318	0.682	0.190	0.0082	0.98	5.60	0.45	0.0104** (0.00004)	0.00358** (0.0003)
	N	42.1 (1.20)	0.259	0.741	0.153	0.0035	0.97	6.96	0.16	0.0168* (0.00068)	0.00048** (0.00022)
	NP	29.3 (0.93)	0.381	0.619	0.136	0.0047	0.98	5.00	0.27	0.00824 (0.00104)	0.00188 (0.00004)
ROOTS	Control	51.1 (1.94)	0.482	0.518	0.018	0.004	0.98	8.36	0.83	0.00042 (0.0005)	0.00015 (0.00001)
	P	47.8 (3.08)	0.138	0.862	0.336	0.0039	0.98	8.88	-0.11	0.0021* (0.00023)	0.00115** (0.0002)
	N	55.6 (2.67)	0.195	0.805	0.102	0.0023	0.93	8.27	0.47	0.00176 (0.00019)	0.00006 (0.00003)
	NP	44.3 (2.23)	0.268	0.732	0.052	0.0031	0.97	7.79	1.02	0.00234* (0.00006)	0.00004 (0.00005)

Table 3. Summary of significant effects of site quality and litter quality on decomposition of wild rice shoot and root litter over time and nutrient dynamics in decaying litter. Arrows represents a significant effect ($p < 0.05$ or less), and arrows pointed up imply a positive or increasing trend over time and arrows pointed down imply the effect was negative or a decreasing trend over time. Decay effects are from repeated measures of the fraction of mass remaining over time; N and P leaching effects are from the ANOVA comparing treatments only to the control litter; and N and P fraction are effects from the repeated measures of the fraction of original N, P during the entire decay period. Equal symbols represent no significant effect.

			Decay	N leaching	P leaching	N fraction	P fraction
Site							
Quality	Shoot	N addition	=	=	=	=	=
		P addition	↑	=	=	=	↑
	Root	N addition	=	=	=	=	=
		P addition	=	=	↓	=	↑
Litter							
Quality	Shoot	N enrichment	↓	↑	↓	=	↑
		P enrichment	↑	↑	↑	↓	↓
		NP enrichment	↓	=	=	↓	=
	Root	N enrichment	=	=	=	↓	↑
		P enrichment	↑	↑	↑	↓	↓
		NP enrichment	=	↑	=	↑	↑

Figure 1. Example of applying the fraction of original mass remaining against nitrogen (N) concentration (Eq. 4) in wild rice control litter decaying over 168 days in the laboratory. Dark circles are the initial values at day 0 and were removed from Eq. 4 in the analysis in order to account for a likely leaching phase (arrow) and all remaining values after day 7 (open circles) were recalculated. Immobilization of nitrogen occurs after leaching phase during microbial decay of plant litter.

Figure 2. Decay of wild rice shoot and root litter in site quality experiment (top) over 115 days and litter quality experiment (bottom) over 168 days. Each point equals the mean of 3-4 litterbags with standard error bars. Roots decay slower than stems and shoot litter decay is influenced more by both internal and external nutrient supply.

Figure 3. Fraction of original N or P remaining in wild rice shoot and root litter decaying in the field over 115 days with the addition of either nitrogen (N) or phosphorus (P) (site quality) or of litter from plants enriched with N, P or both prior to decomposition in the laboratory over 168 days (litter quality). Each point represents 3 to 4 litterbags with standard error bars.

Figure 4. Amount of total nitrogen (N) and phosphorus (P) in grams leached by day 9 (site quality) or day 7(litter quality) during wild rice shoot and root decomposition. Each value represents the mean of either three or four litterbags with standard error bars. Significant differences to control litter shown with * $p < 0.05$ or ** $p < 0.001$.

Figure 5. Initial wild rice, *Zizania palustris*, root and shoot litter chemistry variables linearly regressed against fraction of mass remaining at last sampling day, or decay rates (k_i , from the double exponential model Eq.1), after decomposition over 168 days in the laboratory. Linear regression model (lines) shown only for significant correlations ($p < 0.05$). Each point equals mean of 4 (shoot) or 3 (root) replicates with standard error bars, except decay rates equal a single value estimated by the model. Shoot litter mass remaining was consistently correlated to initial chemistry variables that included P or lignin (lignin+acid-solubles:P was also significant; $p = 0.0331$, $r^2 = 0.935$, not shown). The few predictors of root litter mass remaining included initial water soluble concentrations and lignin concentrations.

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APPENDIX

Figure A-1. Fraction of original N or P remaining on the final sampling day for site quality experiment and litter quality experiment. Each value represents the mean of either three or four litter bags with standard error bars. Significant differences to control litter shown with * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure A-2. Concentration of lignin in wild rice, *Zizania palustris*, shoot and root litter decaying in the laboratory over 168 days. Each line represents different nutrient enrichment prior to decomposition. Each point represents 3-4 litterbags with standard error bars.

Table A-1. Linear regression parameters used to predict the amount of net N or P immobilized in wild rice litter decaying over time in both studies. Predicted values were calculated by applying the slopes and y-intercept from the regressions of the original mass remaining and nitrogen concentrations in the decaying litter to a separate function (not shown, see Eq.4) developed by Aber and Melillo (1982).

Mass remaining vs. N						Mass remaining vs P					
Treat		a (y-int.)	b (slope)	r ²	p	N immob (g/g M ₀)	a (y-int.)	b (slope)	r ²	p	P immob (g/gM ₀)
SITE QUALITY											
Shoots	Control	0.82	0.0013	0	0.998	n/a	0.54	6.57	0.78	<0.0001	-0.12
	P	1.284	-0.666	0.673	<0.001	6.13	1.3444	-7.0035	0.61	0.0001	0.64
Roots	N	1.734	-1.264	0.692	<0.001	5.88	0.4524	7.8262	0.66	<0.0001	-0.07
	Control	1.567	-0.695	0.85	<0.001	8.75	0.2201	5.6773	0.70	<0.0001	-0.03
	P	1.84	-0.961	0.873	<0.001	8.76	0.6307	1.1975	0.10	0.206	n/a
LITTER	N	1.496	-0.599	0.845	<0.001	9.26	0.2295	5.649	0.76	<0.0001	-0.04
	QUALITY										
Shoots	Control	1.1679	0.6384	0.6067	<0.001	5.287	1.0179	-6.4251	0.61	<0.0001	0.400
	P	0.9940	-0.436	0.506	<0.001	5.604	0.9353	-4.8278	0.58	<0.0001	0.449
	N	1.3988	0.6945	0.3912	0.001	6.962	1.3426	-27.189	0.44	0.0004	0.164
Roots	NP	1.2820	-0.81	0.27	0.009	5.004	1.204	-13.277	0.51	0.0004	0.270
	Control	1.4108	0.5886	0.7338	<0.001	8.36	1.5751	-7.4198	0.57	0.0012	0.827
	P	1.4596	0.5935	0.8792	<0.001	8.88	-0.4885	6.3901	0.71	<0.0001	-0.115
LITTER	N	1.5871	-0.7531	0.7896	<0.001	8.27	1.3051	-9.0507	0.40	0.0112	0.466
	NP	1.2379	-0.488	0.8632	<0.001	7.79	1.3792	-4.613	0.68	0.0001	1.020

Table A-2. Correlation coefficients and significant values of linear regression of initial litter quality variables from wild rice, *Zizania palustris*, shoot and root litter against decay parameters. Decay parameters included either the fraction of mass remaining (%MR) on the last sampling day of decomposition over 168 days in the laboratory, or to parameters from a nonlinear regression using a double exponential model: labile pool (s_1) and decay rate (k_1) or recalcitrant pool (s_2) and decay rates (k_2). Mean chemistry values were from litter that had been previously enriched with nitrogen, phosphorus or both prior to decay.

%MR- predictors	Shoot Litter		Root Litter	
	r^2	p	r^2	P
Lignin:P	0.980	0.0100	0.383	0.3809
C:P ratio	0.948	0.0261	0.541	0.2646
Lignin+Acid Solubles:P	0.935	0.0331	0.555	0.2552
N:P ratio	0.915	0.0434	0.674	0.1789
% P	0.877	0.0636	0.161	0.5988
% Lignin	0.832	0.0877	0.016	0.8752
% N	0.773	0.1210	0.163	0.5959
Lignin+Acid Solubles:N	0.568	0.2467	0.041	0.7973
C:N ratio	0.566	0.2478	0.257	0.4927
Lignin:N	0.439	0.3378	0.096	0.6902
% Water soluble	0.338	0.4190	0.907	0.048*
% Lignin+ Acid soluble	0.529	0.2730	0.014	0.8830
s1 predictors				
% Water soluble	0.152	0.6100	0.126	0.6450
% Acid soluble	0.956	0.022*	0.004	0.9400
% Lignin	0.044	0.7910	0.670	0.1810
%Lignin+Acid Soluble	0.014	0.6500	0.063	0.7480
s2 predictors				
% Water soluble	0.687	0.1710	0.126	0.6450
% Acid soluble	0.932	0.032*	0.004	0.7630
% Lignin	0.053	0.7690	0.670	0.1810
% Acid soluble + Lignin	0.013	0.8840	0.063	0.7480
k1 predictors				
% Water soluble	0.088	0.7030	0.157	0.6040
% Acid soluble	0.332	0.4240	0.141	0.6240
% Lignin	0.566	0.2470	0.916	0.043*
% Acid soluble + Lignin	0.227	0.5230	0.663	0.1860
k2 predictors				
% Water soluble	0.117	0.6580	0.281	0.4700
% Acid soluble	0.014	0.8840	0.000	0.9960
% Lignin	0.987	0.007*	0.371	0.3910
% Acid soluble + Lignin	0.705	0.1600	0.001	0.9650