Nitrogen to phosphorus ratio of plant biomass versus soil solution in a tropical pioneer tree, *Ficus insipida*

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Abstract

It is commonly assumed that the N:P ratio of a terrestrial plant reflects the relative availability of N and P in the soil in which the plant grows. Here it was assessed for a tropical pioneer tree, *Ficus insipida*. Seedlings were grown in sand and irrigated with nutrient solutions containing N:P ratios ranging from < 1 to > 100. The experimental design further allowed investigation of physiological responses to N and P availability. Homeostatic control over N:P ratios was stronger in leaves than in stems or roots, suggesting that N:P ratios of stems and roots are more sensitive indicators of the relative availability of N and P at a site than N:P ratios of leaves. The leaf N:P ratio at which the greatest plant dry mass and highest photosynthetic rates were achieved was about 11, whereas the corresponding whole-plant N:P ratio was about 6. Plant P concentration varied as a function of the transpiration rate at constant nutrient solution P concentration, possibly due to transpiration-induced variation in the mass flow of P to root surfaces. Transpiration rate varied in response to nutrient solution N concentration, but not to nutrient solution P concentration, demonstrating nutritional control over transpiration by N but not P. Water-use efficiency varied as a function of N availability, but not as a function of P availability.

Résumé

Il est couramment admis que le rapport N:P pour les plantes terrestres reflète la disponibilité relative d'azote et de phosphore dans sol où croît la plante. Ici, le rapport N:P a été évalué pour un arbre tropical pionnier, le *Ficus insipida.* Les semis ont été plantés dans le sable et irrigués avec des solutions nutritives contenant des rapports N:P allant de 1 a 100. Ce concept expérimental a permis une évaluation des réponses physiologiques à la disponibilité d'azote et de phosphore. Le contrôle homéostatique sur le rapport N:P était plus élevé dans les feuilles que dans les tiges ou les racines, ce qui peut signifier que le rapport N:P des tiges et des racines est un indicateur plus sensible à la disponibilité relative d'azote et de phosphore dans un site donné que celui des feuilles. Le rapport N:P de feuilles auquel les taux les plus élevés de masse sèche et de photosynthèse ont été obtenus était d'environ 11 alors que le rapport N:P pour l'ensemble de la plante était d'environ 6. La concentration de phosphore dans les plantes variait en fonction du taux de transpiration à des niveaux constants de concentration de phosphore dans la solution nutritive, ce qui est possiblement dû à des variations dans le débit de phosphore à la surface des racines provoquées par la transpiration. Le taux de transpiration variait en fonction du niveau de concentration de l'azote dans la solution nutritive, mais pas à celui du phosphore, indiquant un contrôle nutritionnel sur la transpiration par l'azote mais pas par le phosphore. L'efficacité de l'utilisation de l'eau variait en fonction de la disponibilité de l'azote mais pas en fonction de celle du phosphore.

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Chapter One: Introduction

The concentration of nitrogen and phosphorus in plant biomass, [N] and [P], are determined by the relative nitrogen and phosphorus uptake by plants, carbon assimilation [C], and the losses of C, N and P through turnover, leaching, exudation, herbivores and parasites (Chapin & Shaver 1989; Aerts & Chapin 2000; Eckstein & Karlsson 2001). As N and P uptake by plants is intricately linked to availability of these nutrients within the growing medium, the use of nutrient concentrations in plant biomass as indicators of nutrient availability and limitation has been the subject of growing interest. Recent studies in the domain have primarily addressed: 1) whether nutrient concentrations in plants are mainly determined by species' traits or by nutrient availability (Güsewell & Koerselman 2002; Güsewell 2004); 2) whether plant species have similar or different relative nutrient requirements (Güsewell & Koerselman 2002; Tessier & Raynal 2003; Niinemets & Kalevi 2005; Townsend *et al.* 2007; Craine *et al.* 2008) and 3) whether the N:P ratio provides a more appropriate indication of nutrient limitation (Güsewell 2003; Tessier & Raynal 2003; Güsewell 2004; Niinemets & Kalevi 2005; Townsend *et al.* 2007; Craine *et al.* 2008). This study examines a single tropical pioneer species in order to address a combination of questions 1 and 3 by examining the relationship between soil nutrient solution and plant nutrient status in *Ficus insipida*. Data from this experiment can also serve for future experiments aimed at addressing question 2.

Research on nutrient availability predictors has been shifting away from nutrient concentrations and towards biomass N:P ratios (quotient of N and P) which are not directly influenced by the plant's carbon economy (or rather the ratio is not sensitive to plant growth rates) but reflect the balance between uptake and losses of N and P. Although the effectiveness of N:P ratios in plant biomass as a tool for assessing constraints on plant productivity associated with N and P availability has largely been established (Koerselman &

Meuleman 1996; Aerts & Chapin 2000; Tessier & Raynal 2003), the explicit role of the N:P supply ratio in determining these responses has rarely been considered (Güsewell 2005) and only a few studies in plant ecology have varied the supplies of N and P independently from each other (Shaver & Melillo 1984; Ryser & Lambers 1995; Romero *et al.* 1999; Güsewell *et al.* 2003; Agren 2004; Townsend *et al.* 2007; Craine *et al.* 2008). As a result, little is known about the morphological and physiological traits that are decisive in determining the ability of plants to grow and compete under N- or P-limited conditions.

An early study suggested that N:P mass ratios below 14 indicate limitation by N and those above 16 indicate limitation by P (Koerselman & Meuleman, 1996), however it has also been noted that plants with N:P ratios outside these ranges do not always respond to fertilization with the nutrient indicated to be in limiting supply (Güsewell *et al.* 2003; Craine *et al.* 2008). In addition, significant variations have been observed in N:P ratios among species growing at a common site, presumably with access to similar N and P supplies in the soil environment (Agren 2004; Townsend *et al.* 2007; Cernusak et al. 2010). Some species appear to be most successful at high N:P supply ratios (Kirkham 2001; Tomassen *et al.* 2003), and others at low N:P supply ratios (Woo & Zedler 2002), suggesting that these species respond differently in terms of biomass production, morphology and/or physiology to the relative supplies of N and P (Güsewell 2005). Consequently, a meaningful interpretation of the plant biomass N:P ratio requires a more refined understanding of its environmental and physiological controls.

This study will contribute to the growing literature on biomass N:P ratio as an indicator of nutrient availability but, whereas prior studies have focused on wetland (Güsewell & Koerselmann 2002; Güsewell *et al* 2003; Güsewell 2004; Güsewell 2005;), grassland (Niinemets & Kalevi 2005; Craine *et al.* 2008) or temperate environments (Tessier & Raynal 2003; Agren 2004) , this study provides a unique examination of nutrient response

in a tropical pioneer species. Moreover, continued and in-depth analysis of rapid assessed indicators such as the N:P ratio enables researchers to more confidently distinguish N limitation from P limitation in forest trees, an important distinction for a number of applications in ecology and global change science (Bobbink e*t al.* 1998; Lee & Caporn 1998; Matson 1999; Johnson *et al.* 1999; Brouwer *et al.* 2001; Gordon *et al.* 2001; Limpens *et al.* 2003; Güsewell 2004; Fang *et al.* 2010).

Chapter Two: Theoretical Approach

Literature Review

This section will provide a review of the mechanisms which control nitrogen and phosphorus availability, acquisition and uptake and their physiological importance. The review will then focus on the importance of biomass N:P ratio and the recent insights provided by the application of ecological stoichiometry.

Nutrient Availability

Nitrogen

Nitrogen is present in the soil as a mixture of organic nitrogen compounds (C-NH_2, where C is a complex organic group) or inorganic nitrogen compounds (ammonium (NH_4^+) or nitrate (NO_3^-)). With the exception of legumes and actinorhizal plants which develop nitrogen fixing symbioses to capture atmospheric nitrogen (N_2) and recent evidence which suggests plants can also utilize organic nitrogen in the form of amino acids and soluble proteins (Näsholm *et al.* 2009), plants acquire N from the soil in its inorganic form. Ammonium ions bind to the soil's negatively-charged cation exchange complex (CEC) and behave much like other cations in the soil. Nitrate ions, however, do not bond or bond very weakly to the soil solids because they carry negative charges and so exist dissolved in the soil water or precipitated as soluble salts under dry conditions. This lack of bonding or ease of access generally results in nitrate being the predominant source of nitrogen for plants (Touraine 2004) but external factors such as soil pH and rainfall will also predict nitrate accessibility.

Organic nitrogen compounds typically accounts for 95-99% of the potentially available nitrogen in the soil and are found in organic residues or in living soil organisms (Hodge *et al.* 2001) but can very according to the pool where the nitrogen is found. Organic

nitrogen compounds can be converted to ammonium (NH_4^+) by soil microorganisms through mineralisation. Bacteria can then oxidize ammonium via NO_2^- to NO_3^- in a process known as nitrification. Microbes also utilize and immobilize inorganic nitrogen, sometimes resulting in the depletion of the nitrogen available to plants if adequate carbon is available to support the microbial biomass. The level of competition between plants and microbes for soil nitrogen is complex due to the multiple pathways through which N cycles (Hodge *et al.* 2001). Microbial competition combined with predominantly inert organic soil nitrogen often limits supply resulting in plant growth and nitrogen biomass accumulation.

Phosphorus

Although phosphorus is widely distributed in nature, P is not found by itself in its elemental form. Elemental P is extremely reactive and will combine with oxygen when exposed to air and will exist as a phosphate; the simplest phosphate being orthophosphate (PO_4^{-3}).

Phosphate ions react readily with the clay fraction of the soil and ultimately may precipitate out with iron and aluminium in acidic soils and calcium in basic soils in a process called “fixation.” Fixation prevents the leaching of phosphorus, but it also changes it into a form which cannot be accessed by plants. In natural systems more than 80% of phosphorus becomes immobile and unavailable for plant uptake because of adsorption, precipitation, or conversion to the organic form (Holford 1997). Like nitrogen, the short supply of inorganic phosphate often limits plant growth and biomass accumulation.

Nutrient Acquisition

Nitrogen

Nitrogen is the mineral nutrient most frequently limiting to plant growth and considerable attention has therefore been paid to the costs and benefits of nitrogen acquisition

and use (Chapin *et al.* 1987). Plants absorb nitrogen from the soil as both NH_4^+ and NO_3^- ions, but because nitrification is so pervasive, the uptake of the nitrate ion (NO_3^-) by plant roots is the main pathway for entry of nitrogen (Touraine 2004). Since NO_3^- uptake is the bottleneck between the source in the soil solution and the assimilatory pathway in plant cells, it plays a key role in the whole plant N nutrition (Güsewell 2005). The uptake of NO_3^- and its subsequent reduction in plant cells are not only limited by nitrogen availability and the plant's energy demands but also depends on the surface area and structure of the plant's root system (Touraine 2004). This explains why plants in nitrogen limited environments generally exhibit higher root to shoot ratios than plants whose growth is not limited by nitrogen availability.

Only a limited proportion of the root system may actually be effective in the uptake of nitrogen (Robinson 2001) and thus the acquisition of N depends on the distribution of the particular roots active in N uptake within the soil. Rooting depth also determines the ability of the plant to intercept N, particularly NO_3^- during periods of leaching (Gastal & Lemaire 2002). Furthermore, the architecture of the root system determines the volume of soil foraged by roots and thus the nutrients acquired. Its associated construction costs, however, are an important consideration. For example, fine roots have a higher surface area to volume ratio than thick roots and thus require less C for construction per unit root length but may be more expensive for maintenance (per unit root weight) (Lambers & Colmer 2005).

To mitigate construction costs in an environment where nitrogen in the soil is extremely heterogeneous on both a spatial and a temporal scale, roots tend to proliferate in localized areas within soil of high nitrogen content (Drew & Saker 1975; Granato & Raper 1989) and thus specific portions of the root may be exposed to high N concentrations while other parts of the root system are ineffective in N uptake. Plants may sense the soil N concentrations with specific sensors (Malamy & Ryan 2001) and respond to these localised

patches of NO_3^- by preferential lateral root proliferation within the nutrient-rich zones (Drew & Saker 1975).

Transpiration is also related to nitrogen acquisition. Transpiration consumes > 100 moles H_2O per mole CO_2 acquired by photosynthesis, accounting for 13% of global annual terrestrial evapotranspiration (Hack *et al*. 2006). Transpiration is generally considered to be a wasteful but unavoidable consequence of photosynthesis (Kramer & Boyer 1995), occurring because water is lost when stomata open for CO_2 uptake. Transpiration, however, creates a gradient which also facilitates the uptake of nutrients from the suspended soil solution. Thus stomatal pore regulation may be tied not only to photosynthesis but may also be responsive to nutrient limitation; functioning in root to shoot solute transport (Tanner & Beevers 2001) and in driving nutrient movement through the soil to the root. This uptake mechanism is referred to as mass flow but plants can also rely on diffusion from the soil solution to obtain nutrients (Phillips 1976). Generally NO_3^- will reach the root through mass-flow whereas $H_2PO_4^-$ will reach the root through diffusion (mass-flow accounting for ~5% of acquisition). In nitrogen, the importance of mass-flow versus diffusion for nutrient uptake depends on water flow and nutrient concentration of the soil solution. Increased water flux by the plant reduces the nutrient depletion zone that may otherwise develop in the rhizosphere as a consequence of active nutrient uptake (Barber & Cushman, 1981). Mass-flow of nutrients may be especially important for plants with low root densities, providing a mechanism for accessing nutrients in the absence of a massively proliferated the root system (Lambers & Colmer, 2005).

This dual role for transpiration in nutrient acquisition may explain why many plants continue to transpire at night. The stomata of many species remain partially open at night (Caird *et al*. 2007) with the result that night-time transpiration accounts for a substantial proportion (e.g. > 10% in paper birch) of water loss (Daley & Phillips 2006). Night time water loss as a result of partial stomatal opening does not reflect a passive leakage of water

from the leaf but is regulated much like daytime water loss and is distinct from and considerably faster than cuticular water loss (Caird *et al*. 2007). This night-time water loss may function in nutrient acquisition through mass-flow (Ludwig *et al*. 2006; Howard & Donovan 2007; Scholz *et al*. 2007). Thus night-time water loss may serve a role in nutrient acquisition and be responsive to nutrient availability, although more evidence is needed for the control of stomatal conductance by nutrient availability, both during the day and at night (Cernusak *et al.* 2010).

Phosphorus

Although several different forms of phosphorus exist in the soil substrate ($H_2PO_4^-$, $H_2PO_2^{-4}$, PO_3^{-4}), it has been found that the dihydrogen form of the orthophosphate ion ($H_2PO_4^-$) is the most readily transported into plant cells (Raghothama 1999).

The uptake of phosphorus poses a problem for plants since the concentration of this mineral in the soil solution is very low but plant requirements of phosphorus are high (Bieleski 1973). Consequently, plants must have specialized transporters at the soil / root interface for extraction of phosphorus from soil solutions, as well as other mechanisms for transporting phosphorus across membranes between intracellular compartments, where the concentrations of phosphorus may be 1000-fold higher than in the external solution (Schachtman *et al*. 1998).

As with nitrogen, plants also respond to heterogeneously distributed phosphorus in the soil by enhancing uptake in localized P-rich patches (Drew & Shaker 1978; Jackson *et al.* 1990). This phenotypic plasticity helps to maximize phosphorus uptake without wasting valuable carbon resources through root construction. For example, an observed 80% increase in uptake by roots growing in enriched soil patches is a clear indication of the physiological plasticity exhibited by plants (Jackson *et al.* 1990). This physiological flexibility of roots is

crucial in compensating for a lack of uptake of phosphorus by major portions of the root system. Unlike nitrogen, however, there is a high degree of correlation between mycorrhizae formation and phosphorus status of soil (Wilcox 1991). Mycorrhizae are considered to be an integral part of the phosphorus absorption since these fungal hyphae greatly increase the volume of soil that plant roots explore (Smith & Read 1997).

Diffusion accounts for the vast majority of phosphorus uptake by plants since phosphorus is largely insoluble and subsequently its concentration in soil solution is very low rendering acquisition through mass flow inconsequential (Chapin *et al* 2002). Consequently, given that there is generally a low availability of phosphorus in the soil and the rate of diffusion of phosphorus into the roots results in a zone of phosphorus depletion around the roots that is not readily replenished by mass flow, plant root geometry, morphology and mycorrhizae associations become vitally important for maximizing phosphorus uptake. This is simply because root systems that have higher ratios of surface area to volume will more effectively explore a larger volume of soil (Lynch, 1995).

This section has only provided a cursory introduction to the mechanisms which control nutrient acquisition since growing conditions in this study were carefully controlled in order to reduce complexity and better assess the relationship between soil solution N:P ratio and plant N:P ratio. As such, plants were grown in a nutrient depleted soil medium with limited cation exchange capacity and no mycorrhizal inoculation.

Physiological Role

Nitrogen

Nitrogen is the most important element which affects plant growth and metabolism (Touraine 2004). Nitrogen is a crucial component in many important structural, genetic and metabolic compounds in plant cells. It is a major component of chlorophyll, the compound by

which plants use sunlight energy to produce sugars (i.e. photosynthesis). It is an essential component of nucleic acids and amino acids; the building blocks of proteins and the genetic material that allows cells to grow and reproduce. Nitrogen is also a component of energy-transfer compounds, such as ATP (adenosine triphosphate) which allows cells to conserve and use the energy released in metabolism (Mengel 2001).

Phosphorus

After nitrogen, phosphorus is the second most frequently limiting macronutrient for plant growth (Schachtman *et al.* 1998). Phosphate is one of the key substrates in energy metabolism and biosynthesis of nucleic acids and membranes (Theodorou & Plaxton 1993). High-energy phosphate, held as a part of the chemical structures of ADP (adenosine diphosphate) and ATP, is the source of energy that drives many chemical reactions within the plant. Photosynthesis also requires large amounts of proteins (notably Rubisco) and proteins are synthesized by P-rich ribosomes (Agren 2004).

Role of plant plasticity and translocation

An important consideration in this experiment is the plastic response of plants and the role of nutrient translocation triggered by limitation. Plants exhibit a strong tendency to maintain constant concentrations of ions such as nitrogen and phosphorus in spite of large fluctuations in the external concentrations of those ions (Glass & Siddiqi 1984). Plastic responses of plants to nitrogen and phosphorus supply can be responsible for up to 50-fold variation in biomass N:P ratios, associated with differences in root allocation, nutrient uptake, biomass turnover and reproductive output (Güsewell 2004). This plastic response to nutrient supply will also determine the ability of species to grow and compete under various nutrient

regimes (Robinson & Rorison 1988; Tilman & Wedin 1991; Reynolds & D'Antonio 1996; Garnier 1998).

Insight can be gained by examining the inter-specific differences caused by nitrogen and phosphorus availability as reflected in differences in plant traits and the physiological mechanisms that determine how efficiently nitrogen and phosphorus are used for growth (Nomura 2004). Nitrogen requirements are largely determined by photosynthetic nitrogen use efficiency (Garnier *et al*. 1995), which varies widely among species because of differences in leaf morphology, internal distribution of N and production of N compounds not involved in photosynthesis (Lambers & Poorter 1992). Variation in phosphorus requirements can be related to rates of internal phosphate recycling (Nanamori *et al*. 2004); the ability to export and transform sugars from chloroplasts under phosphorus deficiency (Nanamori *et al*. 2004); or the availability of alternative phosphorus-saving metabolic pathways for glycolysis and mitochondrial respiration (Vance *et al*. 2003).

Life Span

It is also important to note that studies of N:P ratios are often simplified by focusing on young plants in growth experiments. Young plants consist largely of immature leaves that assimilate and grow simultaneously, so that the demands of N and P are given by the stoichiometry of basic biochemical processes (photosynthesis, respiration protein synthesis, DNA duplication and DNA transcription) (Güsewell, 2004). Young plants will generally take up all nutrients used for growth from the environment, whereas older plants partly use nutrients which they stored previously (e.g. Jonasson & Chapin 1985; Pfadenhauer & Lütke-Twenhöven 1986; Bernard *et al.* 1988). Moreover growth in older plants is restricted to active meristems and their mature leaves are still photosynthetically active but no longer grow, which greatly reduces the P requirements for RNA (Güsewell 2004). Nucleic-acid P can

therefore be mobilized and translocated to young leaves, leading to higher plant-level N:P ratios (Usuda 1995). Interestingly the ability of plants to recycle N and P also differs: only 50-60% of nitrogen, but as much as 80-90% of phosphorus can be reabsorbed from the above-ground biomass (Aerts & Chapin 2000).

Ultimately since this experiment investigates the relationship between plant N:P ratio and nutrient acquisition rather than nutrient acquisition with nutrient conservation/ translocation, a short experimental time frame was selected.

Uptake and the response and implication of deficiency and excess

Uptake

Nitrogen and phosphorus uptake rates are controlled by whole-plant signalling mechanisms (Imsande & Touraine, 1994; Raghothama, 1999; Forde, 2002). This regulation is both positive and negative: N-deficient plants increase the rate of nitrogen uptake and reduce the rate of phosphorus uptake while P-deficient plants do the opposite (Aerts & Chapin, 2000).

At lower nutrient supply, plants not only grow more slowly than at higher nutrient supply but they also increase their biomass allocation to roots (Poorter & Nagel 2000) and reduce the nutrient concentrations of their biomass (Aerts & Chapin 2000). At high nutrient supply, the opposite phenotypic responses enable the plants to improve their carbon gain and to compete more effectively against other plants when light becomes limiting (Garnier 1998; Hirose & Bazzaz 1998; Schieving & Poorter 1999; Poorter & Nagel 2000). This type of phenotypic plasticity has been observed in response to nitrogen supply under N-limited conditions and in response to phosphorus supply under P-limited conditions (Wilson 1988; Ingestad & Ågren 1991; Ericsson 1995; Ryser *et al.* 1997). This similarity of effects suggests that some plant traits respond to nutrient supply in a general way, rather than specifically to

the supply of either N or P (Güsewell 2005) further supporting the application of a relative indicator such as the N:P ratio to nutrient limitation studies.

Nitrogen

Biomass allocation to roots increases in response to deficiency of both nitrogen and phosphorus, but the effect of nitrogen is usually stronger (Andrews *et al.*1999; De Groot *et al.* 2003). Nitrogen deprivation causes an increase in the proportion of photosynthate translocated to the root which results in a decline in the shoot:root ratios (Rufty *et al.* 1988). In periods of excess, evidence supports the existence of plant signalling elicited by $NO3^-$ accumulation that represses root growth and in turn determines carbon allocation in the roots and stems (Scheible *et al.* 1997; Tranbarger *et al.* 2003). This signaling and subsequent downregulation by feedback inhibition is thought to occur when nitrate accumulates in roots instead of being exported to shoots (Gniazdowska *et al.* 1999), or when amino acids not used for growth cycle back from shoots to roots (Imsande & Touraine 1994). Plants with a high N:P ratio normally allocate less biomass to roots than plants with same growth rate but a low N:P ratio (Güsewell *et al*. 2003a).

A nitrogen-deficient plant is generally small and develops slowly because it lacks the nitrogen necessary to manufacture adequate structural and genetic materials. Its leaves are typically pale green or yellowish because it lacks adequate chlorophyll. Older leaves often become necrotic and die as the plant translocates nitrogen from less important older tissues to more important younger ones (Mengel 2001).

Phosphorus

Modification of root growth and architecture is also a well-documented response to phosphorus starvation (Lynch 1995, Lynch 1997). An increase in the root-shoot ratio under

phosphorus starvation is a hallmark response to phosphorus deficiency as this modification enhances the total surface area available for soil exploration and acquisition of nutrients (Raghothama 1999). Phosphorus uptake can also be enhanced in response to phosphorus deficiency through other mechanisms, such as the activation of specialized carrier proteins (Schachtman *et al.* 1998); the formation of root hairs or cluster roots (Lamont 2003; Shane *et al*. 2003); the exudation of enzymes or acids (Schachtman *et al.* 1998; Kamh *et al.* 1999; Dakora & Phillips 2002); and increased mycorrhizal infection (Jayachandran *et al.* 1992). Recently, specific genes have been identified in tomato that are upregulated both by increased nitrate supply and by phosphorus deficiency, suggesting the possibility of a direct relationship between N:P supply ratios and regulation mechanisms (Wang *et al.* 2001). During periods of excess phosphorus, uptake is down-regulated in response to high phosphate concentration in the phloem (Marschner *et al.* 1996; Schachtman *et al*. 1998). As an alternative to reduced uptake, storage of phosphorus as polyphosphate in stems, roots and seeds avoids toxic effects and can support growth at other times (Handreck 1997).

When phosphorus is limiting, the most visually striking effects are a reduction in leaf expansion and leaf surface area as well as the number of leaves produced. Shoot growth is more affected than root growth, which leads to a decrease in the shoot root dry weight ratio (Ericsson 1995). Root growth is also reduced by phosphorus deficiency leading to a reduced root mass. Generally, inadequate phosphorus slows the processes of carbohydrate utilization, while carbohydrate production through photosynthesis continues unaffected. This results in a build-up of carbohydrates and the development of a dark green or purplish leaf colour that is characteristic of phosphorus deficiency (Mengel 2001). Since phosphorus is readily mobilized in the plant, when deficiency occurs the phosphorus is translocated from older tissues to active meristematic tissues, resulting in foliar deficiency symptoms appearing only in the older (lower) portion of the plant (Berry 2006).

Importance of N:P ratio

If two or more nutrients can potentially be in short supply, their availability relative to each other is likely to determine which of them is limiting (Koerselman & Meuleman 1996). The ratio of N to P availability should therefore determine which of them is limiting, and thus the N: P ratio, a variable which reflects this relative availability, would be indicative of the limiting nutrient (Koerselman & Meuleman 1996).

Recent studies have employed the N: P ratio to identify thresholds of nutrient limitation (Koerselman & Meuleman 1996; Verhoeven *et al*. 1996b; Aerts & Chapin 2000; Giisewell & Koerselman 2002). Moreover, using the N:P ratios of plant biomass as indicators of nitrogen or phosphorus limitation, various studies have suggested that shifts in limitation lead to changes in plant traits, vegetation composition and species diversity (Koerselman & Meuleman, 1996; Verhoeven *et al*., 1996a; Roem & Berendse, 2000). Plant N:P ratios have also been used to describe functional differences between naturally N- or P-limited plant communities and their responses to environmental change or human management (Verhoeven *et al*. 1996b; Bedford *et al*. 1999; Matson *et al*. 1999; Olde Venterink *et al*. 2003).

Pioneering work by Güsewell proposed that the N:P ratio is more closely related to nutrient availability and is thus a better indicator of the type of nutrient limitation than nitrogen and phosphorus concentrations. Güsewell showed that 1) the N:P ratio in plants varied relatively less among species than individual N and P concentrations 2) the N:P ratio also varied less in response to the total supply of N and P, and 3) the N:P ratio varied more in response to differences in the relative availability of N and P (Güsewell 2002). Ranges were proposed to identify nutrient limitation. When plant N:P was <14, N was considered limiting, when plant N:P was >16, P was considered limiting and in between, N and P were co-limiting (Güsewell 2002; Craine *et al.* 2008). Güsewell (2004) later proposed a broader

range of ratios for co-limitation of plant communities, stating that 'it appears that biomass production is most likely to be enhanced by N fertilization in vegetation with N:P ratio <10 and by P fertilization in vegetation with N:P ratio >20, whereas within this range, the effects of fertilization are not unequivocally related to N:P ratios'.

Studies on N:P ratios have also drawn attention to the fact that there is often no clear-cut distinction between N and P limitation, but a gradient with varying degrees of N and P deficiency (Sinclair *et al.* 1997; Craine *et al.* 2008). N:P ratios in individual plant parts may depend additionally on internal nutrient translocation, particularly when the plant is in a reproductive phase as phosphorus will migrate to the seed (Güsewell 2004). This translocation of nutrients between tissues is a further complication in understanding the critical N:P in autotrophs. The relative contribution of different tissues will change with the size of the plant and the whole-plant critical N:P will then be changing not only with growth rate but also with plant size. This has led some to believe that the possibility of truly identifying the critical N:P in any given situation may therefore be limited and a single critical N: P might thus serve as only an approximate substitute (Agren 2004).

This experiment therefore investigates the relationship between plant and soil N:P ratios in young plants, which generally take all their nutrients used for growth from the environment, to reduce the complexity which results from the internal translocation of nutrients in mature plants.

Ecological Stoichiometry

Ecological stoichiometry is the study of the balance of energy and multiple chemical elements in ecological interactions (Sterner & Elser 2002). Sterner and Elser suggested a method for analysing the extent of homeostatic regulation that an organism exerts with respect to a resource that it consumes. The method involves plotting the consumer

stoichiometry on the *y*-axis of a graph against resource stoichiometry on the *x*-axis. The relationship between consumer and resource stoichiometry can then be described as

$$\frac{dy}{dx} = \frac{1}{H}\frac{y}{x}, \qquad (1)$$

where H is a regulatory coefficient greater than one that describes the extent of homeostasis. As H approaches infinity, the change in consumer stoichiometry as a function of a change in resource stoichiometry approaches zero, or perfect homeostasis. Equation (1) can be integrated to give

$$y = cx^{\frac{1}{H}}, \qquad (2)$$

where *c* is a constant. Visualization and analysis of H can then be simplified by linearizing equation (2) using logarithms:

$$\log y = \log c + \frac{1}{H}\log x . \qquad (3)$$

The regulatory coefficient, H, can thus be calculated as the inverse of the slope of the relationship between consumer stoichiometry and resource stoichiometry plotted on a log-log scale.

The extent to which the plant N:P ratio reflects that of the soil solution is a function of the homeostatic regulation exerted by the plant over its N:P stoichiometry. The degree of regulation depends on overall nutrient supply and on light intensity. Homeostatic regulation is needed because a reduction of nitrogen and phosphorus below the minimum required to maintain cell function causes the senescence of tissues (Batten & Wardlaw 1987), whereas an excessive increase of N and P leads to toxic effects (Loneragan *et al*. 1979; Loneragan *et al*.

1982; de Graaf *et al*. 1998; Lucassen *et al*. 2002). Various growth experiments with herbaceous plants grown at N:P supply ratios between 1 and 100 revealed regulatory coefficients between 1.7 and 4.6 (recalculated from Shaver & Melillo 1984; Ryser & Lambers 1995; Güsewell et al. 2003a; Güsewell 2004). Regulation of N:P ratios in plants is therefore stronger than in algae or fungi ($H = 1–2$, Rhee 1978; Sterner & Elser 2002) but weaker than in animals or bacteria ($H = 3$, Sterner & Elser 2002; Jaenike & Markow 2003; Makino *et al*. 2003).

This experiment will examine the homeostatic control exhibited by immature *Ficus insipida* plants by examining the correlation between soil and plant N:P ratios to see whether it conforms with published findings.

Scope of Study

In this study, the relationship of the biomass N:P ratio of a tropical pioneer tree, *Ficus insipida* Willd. (Moraceae), to variation in the soil solution N:P ratio was tested. Leaves, stems, and roots were each assessed independently, to determine whether organ-specific variation exists in the extent of homeostatic control over biomass N:P. In addition, the experimental design allowed for investigation of the responses of several physiological processes to variation in N and P availability in this species. These processes included growth, biomass allocation, photosynthesis, stomatal conductance, transpiration, and water-use efficiency.

Chapter Three: Methodology and Data

Plant material and experimental treatments

The experiment took place at the Smithsonian Tropical Research Institute, Santa Cruz Experimental Field Facility, Gamboa, Panama. The site is located at 9°07' N, 79°42' W, and is at an altitude of 28 m above sea level (Map 1). Seeds of *Ficus insipida* were collected from the forest floor in the Panama Canal watershed. Seeds were germinated in trays containing a commercial potting mix in July 2007. Following germination, 70 seedlings were transplanted into 7 L pots, with one seedling in each pot. Each pot contained a volumetric mixture of 95% non-calcareous sand and 5% soil from the A horizon of a nearby forest. The sand was supplied locally and had a Mehlich-3 extractable P concentration (Mehlich 1984) of 4.8 mg P kg^{-1} sand, whereas the topsoil contained 26.4 mg P kg^{-1} soil. It is expected that the high sand content of the mixture would reduce the cation exchange capacity, such that [N] and [P] of the soil solution in the pots would be similar to those of nutrient solutions fed to the plants.

The pots were placed on plastic tables under a translucent rain shelter, which elevated them approximately 2 m above the concrete surface below the shelter. The shelter reduced incoming photon flux density (PFD) by approximately 20%. The plants were rotated around the shelter every two weeks. Nutrient solution treatments were initiated after an adjustment period of one week. Both the above ground and below ground biomass of the plants were harvested after nine weeks of treatment with nutrient solutions, on 14 November 2007.

The seven nutrient solution treatments, including an additional null or control treatment, were applied to eight pots each for a total of 64 experimental pots. Seedlings from the six remaining pots were harvested to estimate the seedling dry mass at the beginning of the experiment (0.013 g) and the initial leaf area (2.81 cm^2). These pots later served as controls for transpiration measurements.

The seven nutrient solution treatments contained varying amounts of N and P (Table 1), based on the treatments described by Wong (Wong *et al.* 1985). A micronutrient solution was also applied (Table 2). Base solutions were prepared using distilled water. For application to plants, base solutions were diluted with rainwater collected from the roof of the rain shelter. Rain collected on Barro Colorado Island, approximately 20 km from the experimental site, during 2008 had [N] of < 10 μM and [P] < 0.3 μM (B.L. Turner & A. Zimmermann, unpublished). The N was supplied to the nutrient solutions as nitrate and P as phosphate. The amounts of other chemicals in the solutions were equalized across the treatments, to avoid any confounding effects (Table 1). Each pot received 1 L of nutrient solution, including 1 ml of micronutrient solution, three times per week; on Monday, Wednesday and Friday.

After harvest, the leaf area of each plant was determined with an LI-3100 Leaf-Area Meter (Li-Cor Inc., Lincoln, NE, USA). Plants were oven-dried at 70°C. Dry mass of leaves, stems, and roots was determined separately for each plant. The final plant dry mass of control plants, irrigated with only rain water, was 0.19 ± 0.15 g (mean ± 1SD), indicating that nutrient availability in the absence of added nutrient solution was very low.

Gravimetric transpiration measurements

Prior to harvest, the pots were weighed for a 5-day period, between 9 and 13 November 2007, in order to estimate transpiration rates on a whole-plant basis. The pots were weighed with a balance that had a capacity of 60 kg and resolution of 2 g (Sartorius EA60EDE-1, Thomas, Swedesboro, NJ, USA) at dawn and dusk on each day. The pots were watered to field capacity each day after the dusk measurements, either with rainwater or the nutrient solution, depending on the day. To account for evaporation from the soil surface, water loss from the control pots without plants was measured and this value was then

subtracted from the water loss of pots with plants to calculate plant transpiration. Mean transpiration rates were expressed on a leaf area basis by dividing the mean water loss per plant by the leaf area determined at plant harvest.

Leaf gas exchange measurements

Gas exchange of the youngest, fully-expanded leaf of each plant was measured between 09:00 and 11:00 local time on 29 and 30 October 2007 with a Li-6400 portable photosynthesis system (Li-Cor Inc., Lincoln, NE, USA). Measurements were made under irradiance of 1500 μmol photons m^{-2} s^{-1} supplied by an artificial light source (6200-02B Li-Cor). Mean leaf temperature during gas exchange measurements was 30.6 ± 0.4°C (mean ± 1SD), and leaf-to-air vapour pressure difference was 0.7 ± 0.1 kPa.

Isotopic and elemental analyses

Leaf, stem, and root dry matter were ground to a fine, homogeneous powder for elemental and isotopic analyses. The $\delta^{13}C$ of leaf, stem, and root dry matter was measured at the Soil Analysis Laboratory at the Smithsonian Tropical Research Institute with an isotope ratio mass spectrometer (Delta V Advantage, Thermo, Bremen, Germany) coupled by a continuous flow interface to an elemental analyser (Flash HT, Thermo). In addition to isotopic analysis, the C and N elemental concentrations were determined from peak areas obtained from mass spectrometric measurements. Whole-plant $\delta^{13}C$ was calculated by mass balance using the dry mass of each plant organ (leaves, stems, and roots), the C mass fraction, and the $\delta^{13}C$ composition. The $\delta^{13}C$ provides an integrated measurement of c_i/c_a or the measurement of intercellular CO_2 concentrations (c_i) over atmosphere CO_2 concentrations (c_a). That is to say that the $\delta^{13}C$ integrates over the lifetime of the leaf, whereas the gas exchange measurement provides an instantaneous measurement of c_i/c_a . The c_i/c_a provides an estimate of how much

water vapor diffuses out through the stomata for a given rate of photosynthesis. Thus, a low c_i/c_a equates to high water use efficiency, and high c_i/c_a to low water use efficiency.

To measure the P concentration of leaf, stem and root dry matter, approximately 200 mg of oven-dried plant material was digested under pressure in PTFE vessels at 180°C in concentrated HNO_3 for 6 hours. Phosphorus was detected in the digestion solutions by inductively-coupled plasma optical emission spectrometry (Optima 2100, Perkin Elmer, Shelton, CT, USA), with samples evaluated against standards prepared in the same matrix.

Statistical analyses

Analysis of variance was used to assess variation in elemental composition and physiological processes in response to nutrient solution treatments. Where significant variation was detected ($P<0.05$), pair-wise comparisons between treatment means were carried out by Tukey's method to determine which nutrient treatments significantly differed from one another. Where necessary, data were log-transformed or inverse-transformed prior to analysis to meet assumptions of normality and homogeneity of variance. Relationships between continuous variables were assessed using least-squares linear regression. To determine the regulatory coefficient, H, from log-log plots of plant biomass N:P ratios against nutrient solution N:P ratios, geometric mean regression was used to facilitate visual interpretation of the curve. In this case, the primary interest was in interpreting the values of the regression coefficients, and both parameters were measured with error, thereby indicating the use of geometric mean regression (Sokal & Rohlf 1995).

Chapter Four: Empirical Section – Results

Variation in final plant dry mass, leaf area ratio, and root:shoot ratio as a function of nutrient solution [N] and [P] is shown in Fig. 1. The left panels in Fig. 1 (A, C, and E) show responses as a function of variation in nutrient solution [N] with nutrient solution [P] held constant at 1.33 mM. The right panels in Fig. 1 (B, D, and F) show responses to variation in nutrient solution [P] with nutrient solution [N] held constant at 12 mM. Final plant dry mass increased as a saturating function of nutrient solution [N] when nutrient solution [P] was held constant (Fig. 1A). On the other hand, final plant dry mass increased approximately linearly as a function of nutrient solution [P] when nutrient solution [N] was held constant (Fig. 1B). Leaf area ratio, defined as final plant leaf area divided by final plant dry mass, increased with increasing nutrient solution [N] at constant nutrient solution [P] (Fig. 1C), but showed no response to increasing nutrient solution [P] at constant nutrient solution [N] (Fig. 1D). Root:shoot ratio showed a similar pattern, with a strong response to variation in nutrient solution [N] at constant nutrient solution [P] (Fig. 1E), but no response to variation in nutrient solution [P] at constant nutrient solution [N] (Fig. 1F).

Variation in N and P content per plant and in plant N:P ratio as a function of nutrient solution [N] and [P] is shown in Fig. 2. Plant N and P content and plant N:P ratio appeared to increase as saturating functions of nutrient solution [N] at constant nutrient solution [P], as seen in Figs. 2A, 2C, and 2E, respectively. On the other hand, responses to variation in nutrient solution [P] at constant nutrient solution [N] were approximately linear over the range of nutrient solution [P] employed in the study (Figs. 2B, 2D, and 2F).

Variation in the [N] of plant biomass as a function of the nutrient solution [N] and [P] is shown in Fig. 3. The [N] of leaves, stems, and roots increased with increasing [N] of the nutrient solution when [P] was held constant, as seen in Figs. 3A, 3C, and 3D, respectively. The [N] of plant biomass showed little response to variation in nutrient solution [P] when

nutrient solution [N] was held constant (Figs. 3B, 3D, and 3F). However, the stem [N] did increase significantly at the lowest nutrient solution [P], as seen in Fig. 3D.

In contrast to these results, plant biomass [P] showed a strong response to variation in nutrient solution [N] when nutrient solution [P] was held constant (Figs. 4A, 4C, and 4E). For leaves and stems, the response was nonlinear, such that [P] of leaf and stem biomass were lowest at intermediate nutrient solution [N] (Figs. 4A and 4C, respectively). Leaf, stem, and root [P] also increased in response to increasing nutrient solution [P] when nutrient solution [N] was held constant, as seen in Figs. 4B, 4D, and 4F. Across all treatments, the highest [P] of plant biomass occurred when the [P] of the nutrient solution was at its highest value (1.33 mM) and the [N] of the nutrient solution was at its lowest value (0.6 mM).

Comparison of the N:P ratio of plant biomass with that of the nutrient solution is shown in Fig. 5. The plot of leaf N:P against nutrient solution N:P suggests that on a logarithmic scale the relationship between the two was approximately linear over most of the range of nutrient solution N:P employed in the study. At the lowest nutrient solution N:P ratio, however, the relationship departed from linearity (Fig. 5A). For the N:P ratio of stem biomass, on the other hand, the relationship with the nutrient solution N:P on a logarithmic scale was linear over the full range of values (Fig. 5B). The relationship between root N:P and nutrient solution N:P followed a similar pattern to that for stem N:P, but with slight departures from linearity at the highest and lowest nutrient solution N:P ratios (Fig. 5C). Given these patterns, geometric mean regressions were fitted to the log-log plots of plant biomass N:P against nutrient solution N:P, excluding the lowest nutrient solution N:P treatment. With this treatment excluded, the nutrient solution N:P ratios ranged from 1 to 135. Across this range of nutrient solution N:P, the estimated H, describing the extent of homeostatic control over plant biomass N:P, was 6.3 for leaves, 2.5 for stems, and 2.8 for roots. Thus, regulatory control over N:P stoichiometry was considerably stronger in leaves

than that in stems or roots. For whole-plant N:P, the relationship with nutrient solution N:P was approximately linear over the full range of nutrient solution N:P (Fig. 5D). The H calculated for whole-plant N:P was 3.1.

Leaf N:P ratios were higher than stem and root N:P ratios over nearly the full range of values achieved in the study (Figs. 6A and 6B, respectively). However, at the very lowest values, leaf N:P ratios appeared to converge with those of stems and roots. Thus, relationships between N:P ratios of leaves and those of stems and roots were nonlinear (Figs. 6A and 6B). The N:P ratios of stems and roots were generally similar to each other (Fig. 6C), although the relationship also appeared to depart from strict linearity.

The greatest plant dry mass at the conclusion of the experiment was achieved in the nutrient solution treatment that had N:P ratio of 4.1. Nutrient solution treatments with higher or lower N:P ratios resulted in smaller plants (Fig. 7A). When plotted against leaf N:P ratio, final plant dry mass increased with increasing leaf N:P ratio up to a value of about 11, and then decreased as leaf N:P ratio increased further (Fig. 7B). This suggests that the optimal leaf N:P ratio for *Ficus insipida* is around 11. When plotted against whole-plant N:P ratio, final plant dry mass increased up to a value of about 6, and then decreased as whole-plant N:P ratio increased further (Fig. 7C). Therefore, the optimal whole-plant N:P ratio was about half the optimal leaf N:P ratio.

The diurnal whole-plant transpiration rate, measured gravimetrically and expressed on a leaf-area basis, varied significantly in response to variation in the nutrient solution [N] when nutrient solution [P] was held constant. Transpiration was highest at the lowest [N], and decreased as nutrient solution [N] increased (Fig. 8A). On the other hand, gravimetric transpiration showed no response to variation in nutrient solution [P] when nutrient solution [N] was held constant (Fig. 8B).

Photosynthesis, expressed on an area basis, was lower at the lowest nutrient solution [N] than at other nutrient solution [N] (Fig. 9A). Area-based photosynthesis did not vary in response to variation in nutrient solution [P] (Fig. 9B). Stomatal conductance increased as nutrient solution [N] increased from 0.6 to 4 mM, then decreased as nutrient solution [N] increased further (Fig. 9C). Stomatal conductance did not vary in response to nutrient solution [P] (Fig. 9D). The ratio of internal to ambient CO_2 mole fractions, c_i/c_a, decreased in response to increasing nutrient solution [N] with nutrient solution [P] held constant (Fig. 9E). However, c_i/c_a showed no response to variation in nutrient solution [P] with nutrient solution [N] held constant (Fig. 9F).

Variation in whole-plant $\delta^{13}C$ confirmed the trends observed across nutrient solution treatments for c_i/c_a. Whole-plant $\delta^{13}C$ increased with increasing nutrient solution [N] with nutrient solution [P] held constant (Fig. 10A), and showed no response to variation in nutrient solution [P] with nutrient solution [N] held constant (Fig. 10B).

For plants grown at variable nutrient solution [N], photosynthesis expressed on a mass basis increased linearly with increasing leaf [N] (Fig. 11A). The regression equation relating the two was $A_m=9.0N_m+255$ ($R^2=0.68$, $P<0.001$, $n=31$), where A_m is mass-based photosynthesis and N_m is mass-based leaf [N]. For plants grown at variable soil solution [P], photosynthesis also increased linearly with increasing leaf [P] (Fig. 11B), but the relationship was weaker than for leaf [N]. The relationship relating A_m to leaf [P] on a mass basis (P_m) was $A_m=33P_m+500$ ($R^2=0.14$, $P=0.03$, $n=32$). A plot of mass-based photosynthesis against leaf N:P ratio showed that photosynthesis increased to up to leaf N:P of around 12, and then decreased as leaf N:P increased further (Fig. 11C).

Chapter Five: Empirical Section – Discussion

Plant biomass N:P ratios for the tropical pioneer tree *F. insipida* varied as a function of the N:P ratio of the nutrient solutions fed to the plants. Estimates of H, the inverse slope of the log-log relationship between plant N:P and nutrient solution N:P, demonstrated the extent of regulatory control over plant N:P expressed in this species. For nutrient solution N:P ratios ranging from 1 to 135, H for leaf dry matter was 6.3, whereas values for stems and roots were 2.5 and 2.8, respectively. For whole-plant biomass, H for *Ficus insipida* was 3.1. These values can be compared to previously observed values for plant biomass ranging from 1.7 to 4.6 (Güsewell 2004). Therefore, the degree of homeostatic control over the N:P ratio at the whole-plant level in *Ficus insipida* was generally within the range of values observed for other vascular plant species. The N:P ratio of plant biomass reflected variation in the N:P ratio of the soil solution, but the range of N:P observed in plant biomass was limited in comparison to the range of nutrient solution N:P experimentally supplied to the plants. This is similar to the findings of Güsewell and Koerselman that the biomass N : P ratios reflect the relative availability of N and P but that the correspondence is not exact because of homeostatic regulation by plants: 10-fold variation in N : P supply ratios causes only two- to three-fold variation in biomass N : P ratios (Güsewell & Koerselman 2002).

Leaf dry matter showed a higher value for H than stem or root dry matter (Fig. 5). This observation could have important implications for diagnosing relative availabilities of N and P in ecosystems based on measurements of plant biomass N:P ratios. The higher H in leaves indicated that the strength of regulatory control over N:P stoichiometry was stronger in leaves than in stems or roots. This is consistent with the idea that excess N or P taken up from the soil solution could be more effectively stored in stems and roots than in leaves when

the supply of one of these nutrients limits growth but the supply of the other does not (Dyckmans *et al.* 2000; Cernusak *et al.* 2010). This would be a favorable strategy for perennial plants because leaf life spans are likely much shorter than those of stems and coarse roots. Thus, excess N or P stored in leaves would be more vulnerable to loss from the plant by leaf abscission or herbivory than excess N or P stored in stems or coarse roots. Based on these observations in *Ficus insipida*, it is concluded that the N:P ratio of leaf dry matter will likely be a less sensitive indicator of variation in the N:P ratio of the soil solution than that of stem or root dry matter.

The study was conducted on seedlings, the largest of which had a final plant dry mass of around 30 g (Figs. 1A and 1B). The stems of these plants were clearly not woody structures in the same sense as the boles of mature trees. Therefore, it is unknown to what extent results from this study describing organ-specific patterns in N:P stoichiometry can be extrapolated to large trees growing in mature forests. It is possible that the stems of the seedlings studied here could be more analogous to small diameter branches or twigs than to the wood in the main stems of large trees in the way that they regulate their biomass N:P ratios. It is noteworthy, however, that the [P] of bole wood appears to show a high sensitivity to soil fertility (Lloyd *et al.* 2001), presumably reflecting variation in the [P] of the soil solution. Thus, it is also possible that the N:P ratio of the bole wood in mature trees will show a higher sensitivity to the N:P ratio of the soil solution than leaves. Further investigation is required to test this hypothesis.

It is worth highlighting that young plants consist largely of immature leaves that assimilate and grow simultaneously, so that the demands of N and P are given by the stoichiometry of basic biochemical processes (photosynthesis, respiration protein synthesis, DNA duplication and DNA transcription) (Güsewell 2005). Young plants will generally take up all nutrients used for growth from the environment, whereas older plants partly use

nutrients which they stored previously (e.g. Jonasson & Chapin 1985; Pfadenhauer & Lütke-Twenhöven 1986; Bernard *et al.* 1988). The ability of plants to recycle N and P differs: only 50-60% of N, but as much as 80-90% of P can be resorbed from the above-ground biomass (Aerts & Chapin 2000). Moreover growth in older plants is restricted to active meristems and their mature leaves are still photosynthetically active but no longer grow, which greatly reduces the P requirements for RNA (Güsewell 2004).

The optimal leaf N:P ratio for *Ficus insipida* observed in this study was around 11 (Fig. 7B), whereas the optimal whole-plant N:P ratio was around 6 (Fig. 7C). These were the values with which largest final plant dry mass was achieved in the experiment. The optimal leaf N:P ratio determined in this way was similar to the optimal value determined from measurements of leaf photosynthesis. Highest rates of mass-based photosynthesis were achieved at a leaf N:P ratio of around 12 (Fig. 11C). These values are less than N:P ratios ranging from 14 to 16 that have been suggested for plants with balanced supplies of N and P (Koerselman & Meuleman 1996; Aerts & Chapin 2000; Tessier & Raynal 2003; Güsewell 2004). *Ficus insipida* is a fast-growing pioneer tree. Plants with high relative growth rates tend to have lower biomass N:P ratios than plants with low relative growth rates (Agren 2004; Güsewell 2004; Niklas *et al.* 2005; Matzek & Vitousek 2009; Cernusak *et al*. 2010). This could explain why *Ficus insipida* achieved highest growth and photosynthesis at a lower leaf N:P ratio that that expected from studies of some other vascular plant species.

Leaf N:P ratio was higher than the N:P ratio of either stems or roots across the full range of biomass N:P ratios observed in this study, whereas stems and roots were generally similar (Fig. 6). It was previously observed that N:P ratios of leaves were higher than those of stems and roots in herbaceous plants, but not in woody plants (Kerkhoff *et al.* 2006). It seems likely that there would be significant ontogenetic variation in these trends for woody perennial plants. Thus, it is possible that because seedlings were used in this study, inter-

organ variation in biomass N:P ratios was more similar to that previously observed for herbaceous species than for woody species.

The [P] of plant biomass showed a strong response to variation in nutrient solution [N] with the [P] of the nutrient solution held constant (Figs. 4A, 4C, and 4E). This suggests that the [P] of plant biomass was modulated by changes in transpiration rate under variable [N] of the nutrient solution. Transpiration rate varied in response to nutrient solution [N] (Fig. 8A). Expressing transpiration rates per unit dry mass, to make them compatible with [P] per unit dry mass, resulted in a significant correlation between whole-plant [P] and transpiration. The relationship was $[P]=0.59E-3.85$ ($R^2=0.54$, $P<0.001$, $n=25$), where [P] is whole-plant [P] (mg P g^{-1} dry mass), and E is average transpiration rate measured gravimetrically over three days, expressed per unit plant dry mass (μmol H_2O g^{-1} dry mass s^{-1}). This relationship is for plants grown at constant nutrient solution [P].

Solutes can be transported to root surfaces either by diffusion through the soil solution or by mass flow of the soil solution (Barber 1995; Tinker & Nye 2000; McDonald *et al.* 2002; Cramer *et al.* 2008). Mass flow of the soil solution occurs as a consequence of plant transpiration. As a result, some fraction of nutrient transport to root surfaces occurs by mass flow. Under steady-state conditions, an increased rate of water uptake by roots will result in a higher solute concentration at the root surface than would occur at a lower rate of water uptake (Nye & Tinker 1977). This could lead to an increased rate of solute transport across the membranes of root cells due to an increased diffusive flux, an increased convective flux, or an increased rate of active uptake (Dalton *et al.* 1975; Fiscus 1975; Fiscus & Kramer 1975). It is suggested here that transpiration-induced variation in the mass flow of P to root surfaces in *Ficus insipida* caused the correlation between plant [P] and transpiration rate at constant nutrient solution [P]. This was likely facilitated by the relatively high [P] of the nutrient solution, which was 1.33 mM. In contrast, inorganic [P] in natural soils is generally

less than 10 μM (Bieleski 1973; Barber 1995; Schachtman *et al.* 1998). Thus, although mass flow appeared to play an important role in modulating plant [P] in this experiment, it may have little influence over P acquisition in *Ficus insipida* plants growing in natural systems.

Plant transpiration was higher at low nutrient solution [N] than at high nutrient solution [N] (Fig. 8A), consistent with previous observations in *Ficus insipida* and other species (Guehl *et al.* 1995; Livingston *et al.* 1999; Cernusak *et al.* 2007b; Cramer *et al.* 2008). Plant transpiration was measured gravimetrically, and expressed per unit leaf area. Variation in plant transpiration rate in response to nutrient solution [N] resulted from variation in both canopy architecture and stomatal conductance. Self shading was greater at high than at low [N], due to larger canopy development and leaf area ratio at high than at low [N] (Figs. 1A and 1C). Thus, the average intercepted irradiance per unit leaf area was lower in high [N] than in low [N] plants. In addition, stomatal conductance decreased as nutrient solution [N] increased, except for at the lowest nutrient solution [N] (Fig. 9C). These results support the suggestion that plants express some degree of regulation over transpirational water losses in order to control the delivery of N to root surfaces by mass flow (Cramer *et al.* 2009).

An important distinction can be drawn between the interaction observed in this experiment between N and transpiration and the interaction between P and transpiration. In *Ficus insipida*, transpiration rate varied in response to N availability (Fig. 8A). On the other hand, transpiration rate did not vary in response to P availability (Fig. 8B). Plant [P] increased with increasing transpiration rate at constant nutrient solution [P]. This leads to the assumption that plant [N] would also increase with increasing transpiration rate at constant nutrient solution [N], but this cannot be tested in the dataset because plant transpiration did not vary among treatments at constant nutrient solution [N] (Fig. 8B). Thus, the results suggest that variation in N availability in the soil solution caused variation in transpiration,

while accumulation of P in plant biomass was modulated by transpiration, but variation in P availability in the soil solution did not cause variation in transpiration.

Biomass allocation responded to variation in N availability, but did not respond to variation in P availability. This was true for both leaf area ratio and root:shoot ratio (Fig. 1). Similarly, leaf gas exchange characteristics varied in response to N availability, but did not respond to variation in P availability (Fig. 9). These results suggest that variation in soil solution [N] triggered stronger physiological and morphological responses in *Ficus insipida* than did variation in soil solution [P] over the concentration ranges for the two nutrients employed in this study.

The concentration ranges of both N and P employed in the study induced pronounced variations in plant growth, as indicated by variation in final plant dry mass in response to either [N] or [P] when the other was held constant (Figs. 1A and 1B). Thus, growth clearly responded to variation in P availability, whereas biomass allocation and leaf gas exchange did not. The mean final plant dry mass at the highest [N] was less than that at the second highest [N] (Fig. 1A), suggesting the possibility of a toxicity response at the highest [N]. However, the two treatments were not significantly different, further suggesting that the difference in means occurred by chance, rather than as a result of the treatments. In contrast to the saturating response of final plant dry mass to [N], final plant dry mass continued to increase at the highest nutrient solution [P] (Fig. 1B), suggesting that P availability limited growth up to the highest nutrient solution [P] applied to the plants.

Similar result have been observed in greenhouse experiments where plant growth responds mainly or exclusively to N supply (N limitation or N toxicity), while P supply seemed secondary. Plants can adjust to P deficiency better over the short term through a reduction in internal P concentrations (Pérez-Corona & Verhoeven 1996; Güsewell *et al.* 2003a; Güsewell 2004). Conversely, however, P deficiency seems to have a more negative

impact than N deficiency on long-term plant performance through increased mortality (Gotelli & Ellison 2002) and impaired reproduction (Brouwer *et al.* 2001; Güsewell 2004). This phenomenon, however interesting, is better addressed in a longer term experiment which investigates growth and nutrient conservation rather than acquisition.

Plant water-use efficiency is known to increase with increasing [N] in plant biomass, all else being equal (Guehl *et al.* 1995; Livingston *et al.* 1999; Raven *et al.* 2004; Ripullone e*t al.* 2004; Cernusak *et al.* 2007a; Cernusak *et al.* 2007b; Cernusak *et al.* 2010). Some observations also suggest that water-use efficiency increases with increasing P availability, or at least that it increases with relief of P deficiency (Raven *et al.* 2004). Water-use efficiency at a given air vapour pressure deficit (VPD) varies primarily as a function of the ratio of leaf internal to ambient CO_2 mole fractions, c_i/c_a, during photosynthesis (Farquhar & Richards 1984; Farquhar *et al.* 1989b; Hubick & Farquhar 1989; Cernusak *et al.* 2007b; Cernusak *et al.* 2008; Cernusak *et al.* 2009a; Cernusak *et al.* 2009b). Plant $\delta^{13}C$ provides a time-integrated estimate of c_i/c_a (Farquhar *et al.* 1982; Farquhar *et al.* 1989a; Brugnoli & Farquhar 2000). Measurements of c_i/c_a and $\delta^{13}C$ in *Ficus insipida* indicated that water-use efficiency at a given VPD varies as a function of N availability in this species, but does not vary as a function of P availability (Figs. 9 and 10) over the concentration ranges of the two nutrients employed in this study.

Chapter Six: Conclusion

This purpose of this study was to examine whether nutrient concentrations in terrestrial plants were mainly determined nutrient availability and whether the N:P ratio provides an appropriate indication of nutrient limitation for the tropical plant *Ficus insipida.*

The results of the study clearly indicate that the nutrient concentration in *Ficus insipida* is influenced by the nutrient concentrations delivered to the growing medium. The N:P ratio of plant biomass in *F. insipida* reflected variation in the N:P ratio of the soil solution, but biomass N:P spanned a much smaller range than soil solution N:P due to regulatory control by the plant. The extent of homoestatic regulation over biomass N:P was stronger in leaves than in stems or roots. Therefore, it is concluded that stem or root dry matter is likely a more sensitive indicator of environmental variation in the relative availability of N and P than leaf dry matter.

As discussed, the N:P ratio of the soil solution is an important component of environmental control where the extent to which the plant N:P ratio reflects that of the soil solution is a function of the homeostatic regulation exerted by the plant over its N:P stoichiometry. Moreover, as the study of terrestrial N:P ratios evolves, it has been further suggested that measuring the proportions of N and P in the soil microbial biomass may provide yet another tool for assessing nutrient limitation in ecosystems (Cleveland & Liptzin 2007) further reinforcing the interconnectivity of the system.

Understanding that evolution has set biochemical constraints on the chemical composition of living organisms is the value of such studies. In terrestrial ecosystems, increased atmospheric N deposition in recent decades has raised the availability of N relative to other elements (Güsewell 2004). As a result, vegetation has become excessively supplied with N and limited by P or other mineral elements leading to negative impacts of nitrogen saturation (Aber *et al.* 1989; Fenn *et al.* 1998; Falkengren-Grerup & Diekmann 2003).

Knowledge of these constraints and trade-offs in nutrient uptake, regulation and cycling may help in understanding the complex interactions of resource supply and consumer regulation in mediating biodiversity impacts on ecosystem function (Worm *et al.* 2002) and become important when we want to understand the implications of human alterations to element cycles (Hessen *et al.* 2004).

Plant [P] also varied as function of transpiration rate at constant nutrient solution [P], possibly due to transpiration-induced variation in the mass flow of P to root surfaces. Transpiration rate responded to nutrient solution [N], but not to nutrient solution [P], demonstrating nutritional control over transpirational water loss by N, but not P. Measurements of c_i/c_a and $\delta^{13}C$ indicated variation in water-use efficiency as a function of N availability, but not as a function of P availability.

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Tables:

Table 1. Chemical concentrations of nutrient solutions (S-1 to S-7) fed to seedlings of *Ficus insipida*.

Concentration (mM)	Constant P, variable N				Constant N, variable P			
	S-1	S-2	S-3	S-4	S-2	S-5	S-6	S-7
NO_3^-	24	12	4	0.6	12	12	12	12
$H_2PO_4^-$	1.33	1.33	1.33	1.33	1.33	0.53	0.133	0.04
K^+	4	4	4	4	4	4	4	4
Mg^{2+}	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Ca^{2+}	4	4	4	4	4	4	4	4
Na^+	13.33	1.33	1.33	1.33	1.33	0.53	0.133	0.04
SO_4^{2-}	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Cl^-			8	11.4				

Table 2. Chemical concentrations of micronutrient solution fed to seedlings of *Ficus insipida.*

Stock Solution	**mM**	**g/L**
$MnSO_4 \cdot 4H_2O$	0.0100	2.2305
$ZnSO_4 \cdot 7H_2O$	0.0010	0.2876
$CaSO_4 \cdot 5H_2O$	0.0010	0.2497
H_3BO_3	0.0500	3.0915
$Na_2MoO_4 \cdot 2H_2O$	0.0050	1.2098
NaCl	0.1000	5.8440
$CoSO_4 \cdot 7H_2O$	0.0002	0.0562

Figures:

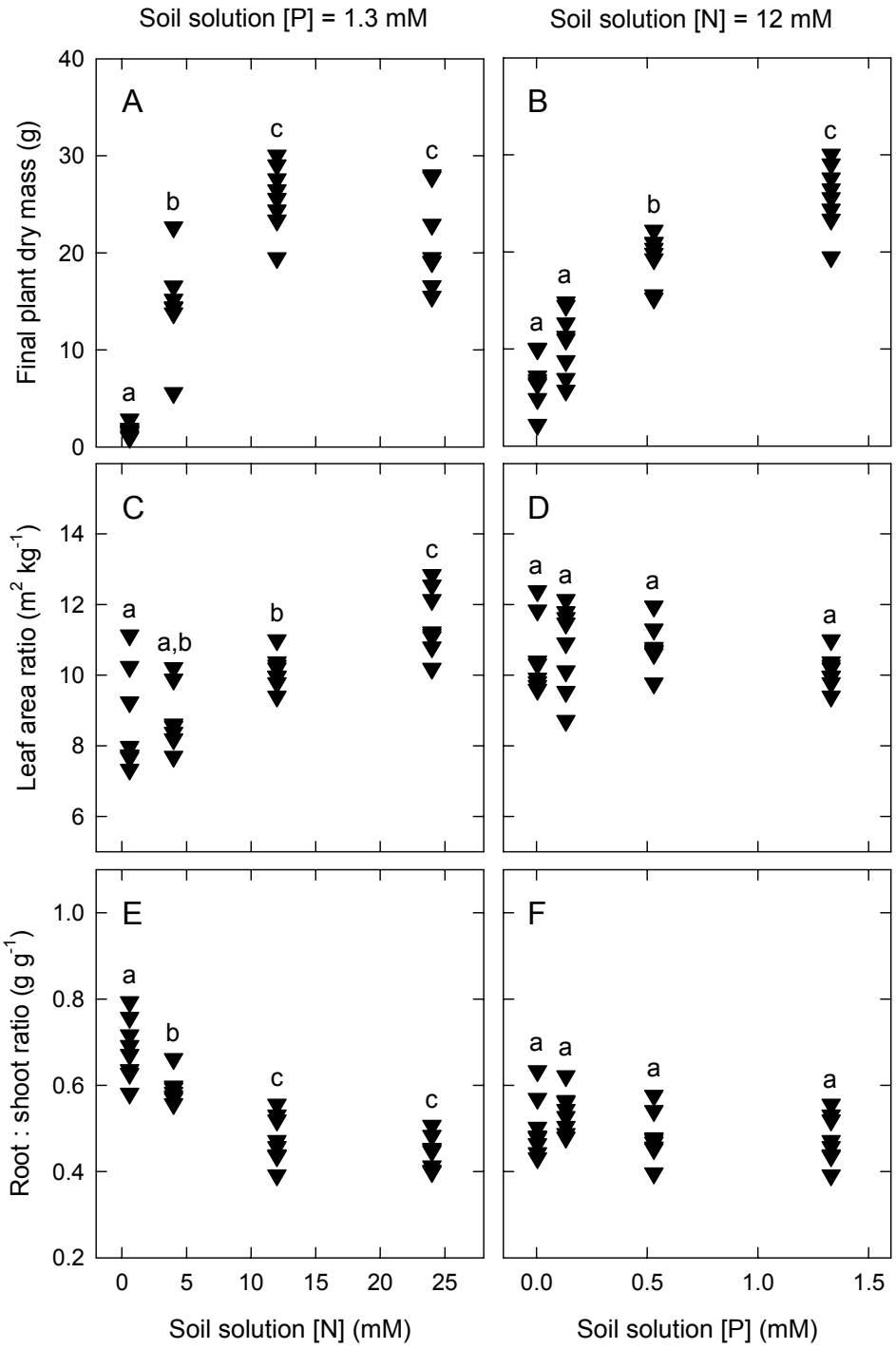


Figure 1. Final plant dry mass (A and B), leaf area ratio (C and D) and root:shoot ratio (E and F) of *Ficus insipida* seedlings plotted against nutrient solution nitrogen and phosphorus concentrations. The left-hand panels (A, C, and E) show variation as a function of nutrient solution nitrogen concentration with nutrient solution phosphorus concentration held constant at 1.33 mM. The right-hand panels (B, D, and F) show variation as a function of nutrient solution phosphorus concentration with nutrient solution nitrogen concentration held constant at 12 mM. Different lower-case letters within a panel indicate significantly different means at $P<0.05$.

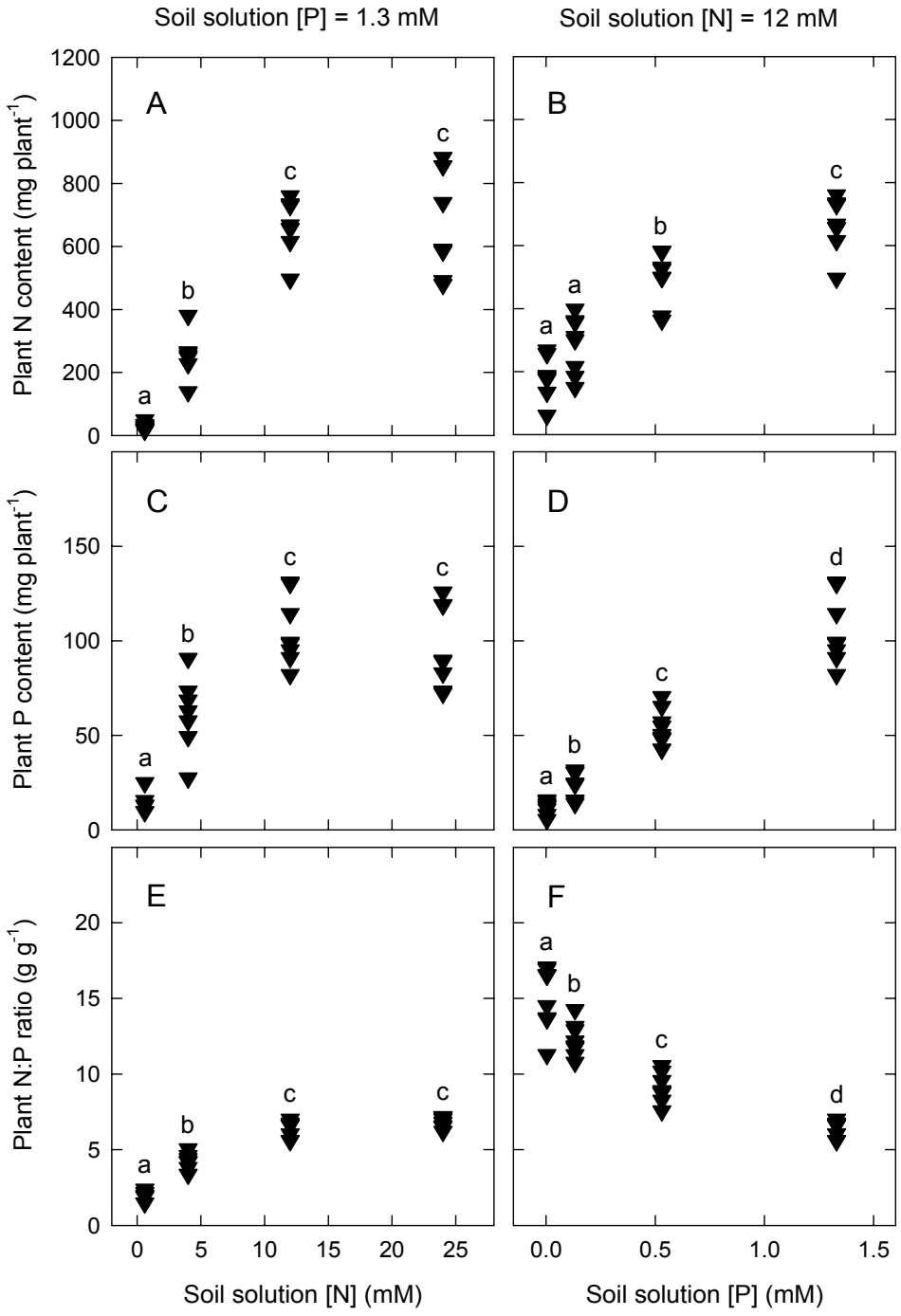


Figure 2. Nitrogen content per plant (A and B), phosphorus content per plant (C and D), and plant nitrogen to phosphorus ratio (E and F) of *Ficus insipida* seedlings as a function of variation in the nitrogen and phosphorus concentration of nutrient solutions fed to the plants. The left-hand panels (A, C, and E) show variation as a function of nutrient solution nitrogen concentration with nutrient solution phosphorus concentration held constant at 1.33 mM. The right-hand panels (B, D, and F) show variation as a function of nutrient solution phosphorus concentration with nutrient solution nitrogen concentration held constant at 12 mM. Different lower-case letters within a panel indicate significantly different means at $P<0.05$.

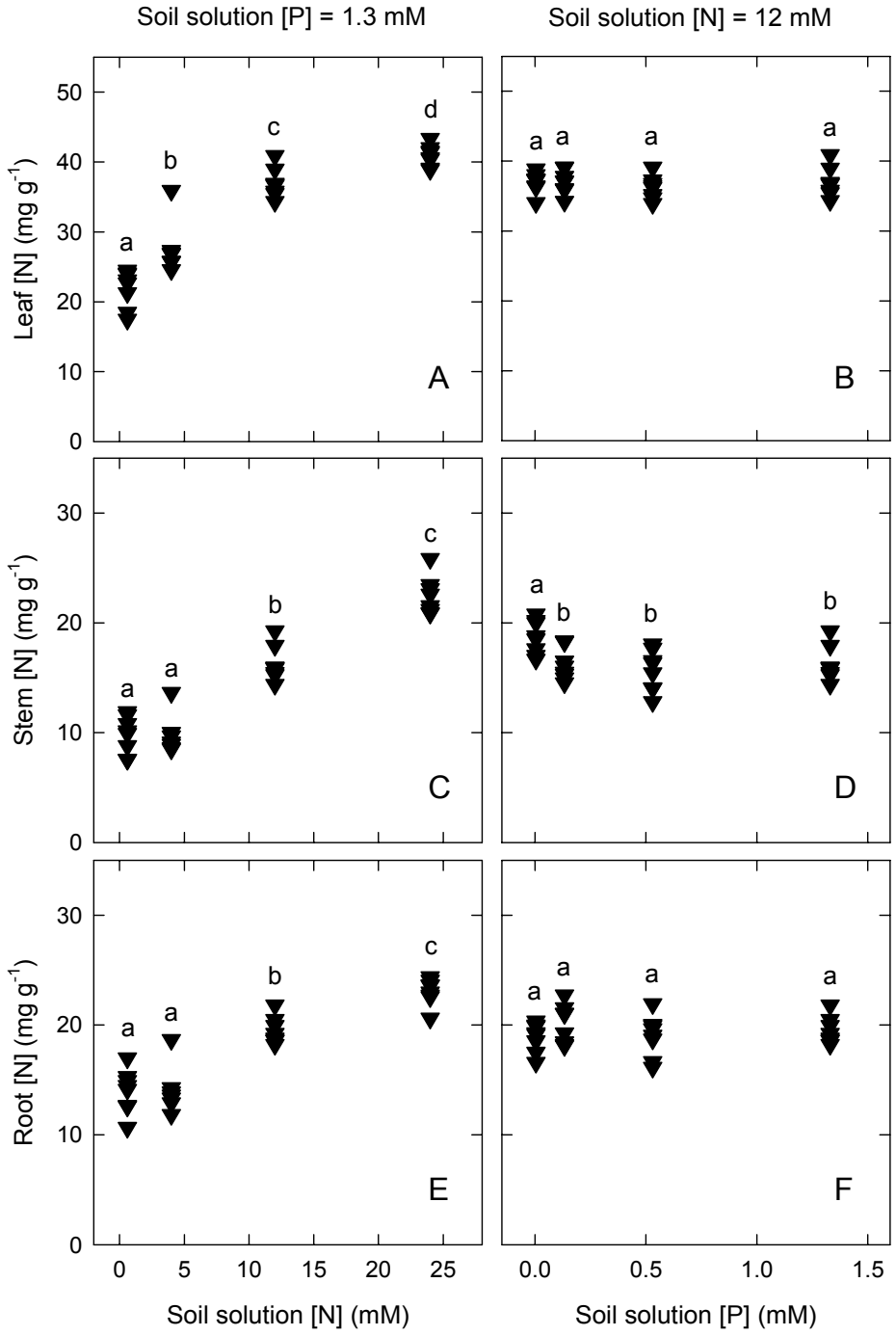


Figure 3. The nitrogen concentration of leaves (A and B), stems (C and D), and roots (E and F) of *Ficus insipida* seedlings as a function of variation in the nitrogen and phosphorus concentration of nutrient solutions fed to the plants. The left-hand panels (A, C, and E) show variation as a function of nutrient solution nitrogen concentration with nutrient solution phosphorus concentration held constant at 1.33 mM. The right-hand panels (B, D, and F) show variation as a function of nutrient solution phosphorus concentration with nutrient solution nitrogen concentration held constant at 12 mM. Different lower-case letters within a panel indicate significantly different means at $P<0.05$.

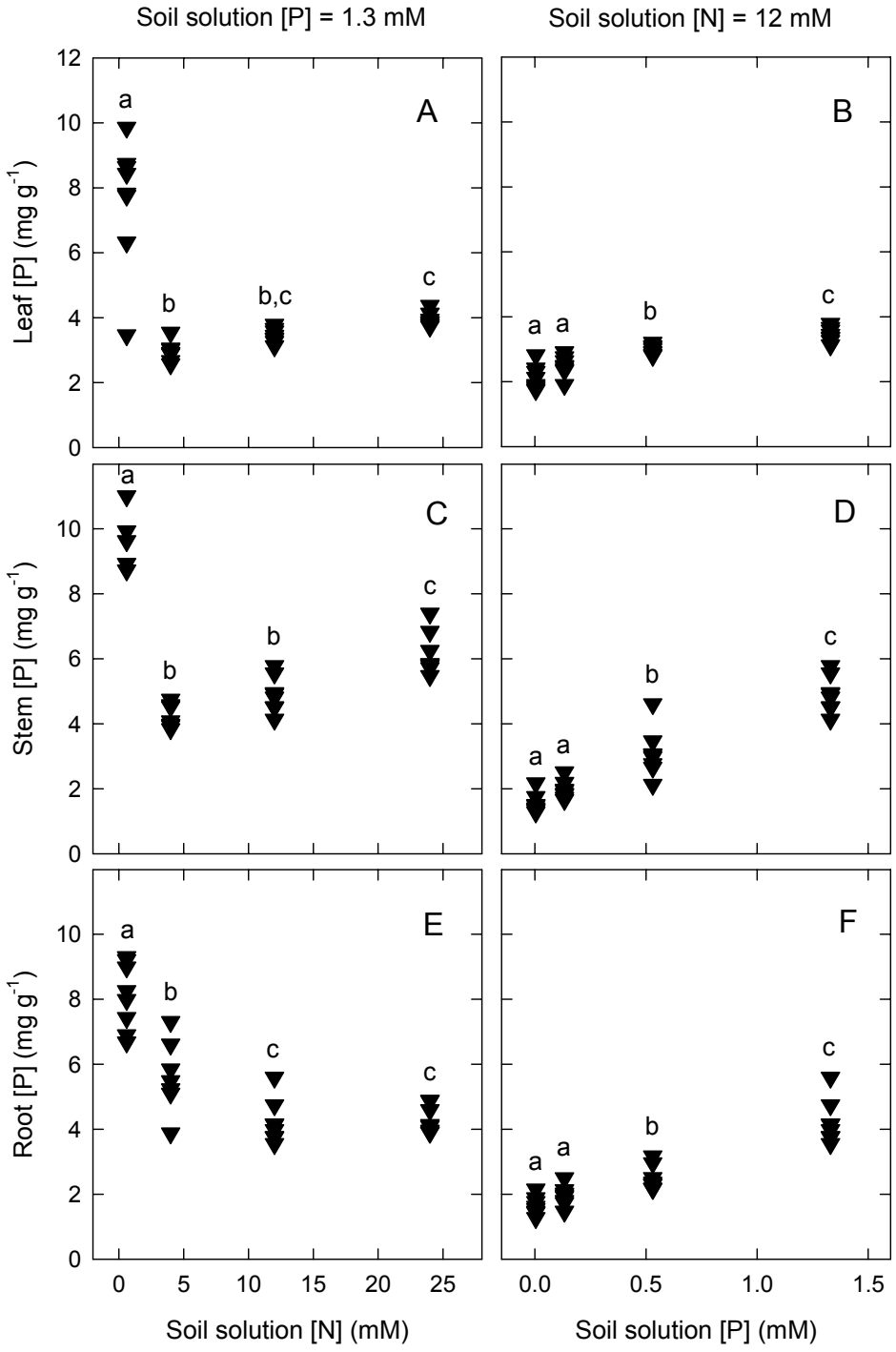


Figure 4. The phosporus concentration of leaves (A and B), stems (C and D), and roots (E and F) of *Ficus insipida* seedlings as a function of variation in the nitrogen and phosphorus concentration of nutrient solutions fed to the plants. The left-hand panels (A, C, and E) show variation as a function of nutrient solution nitrogen concentration with nutrient solution phosphorus concentration held constant at 1.33 mM. The right-hand panels (B, D, and F) show variation as a function of nutrient solution phosphorus concentration with nutrient solution nitrogen concentration held constant at 12 mM. Different lower-case letters within a panel indicate significantly different means at $P<0.05$.

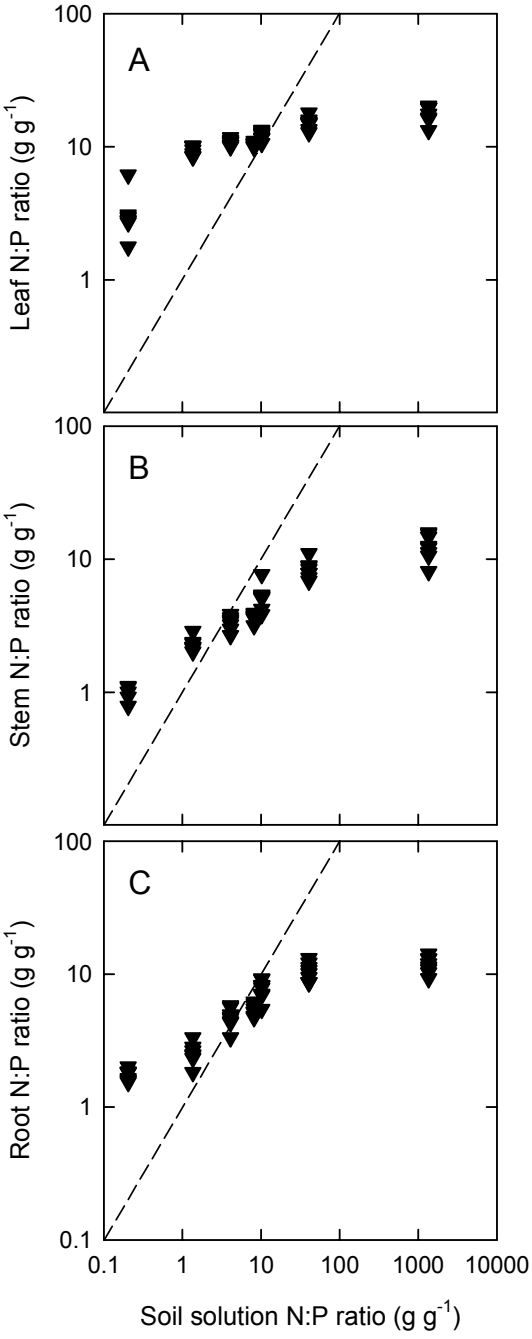


Figure 5. The nitrogen to phosphorus ratio of leaves (A), stems (B), and roots (C) of *Ficus insipida* seedlings plotted as a function of the nitrogen to phosphorus ratio of the nutrient solutions fed to the plants. Both the *x*-axis and *y*-axis are shown on logarithmic scales. The dashed lines represent one-to-one lines.

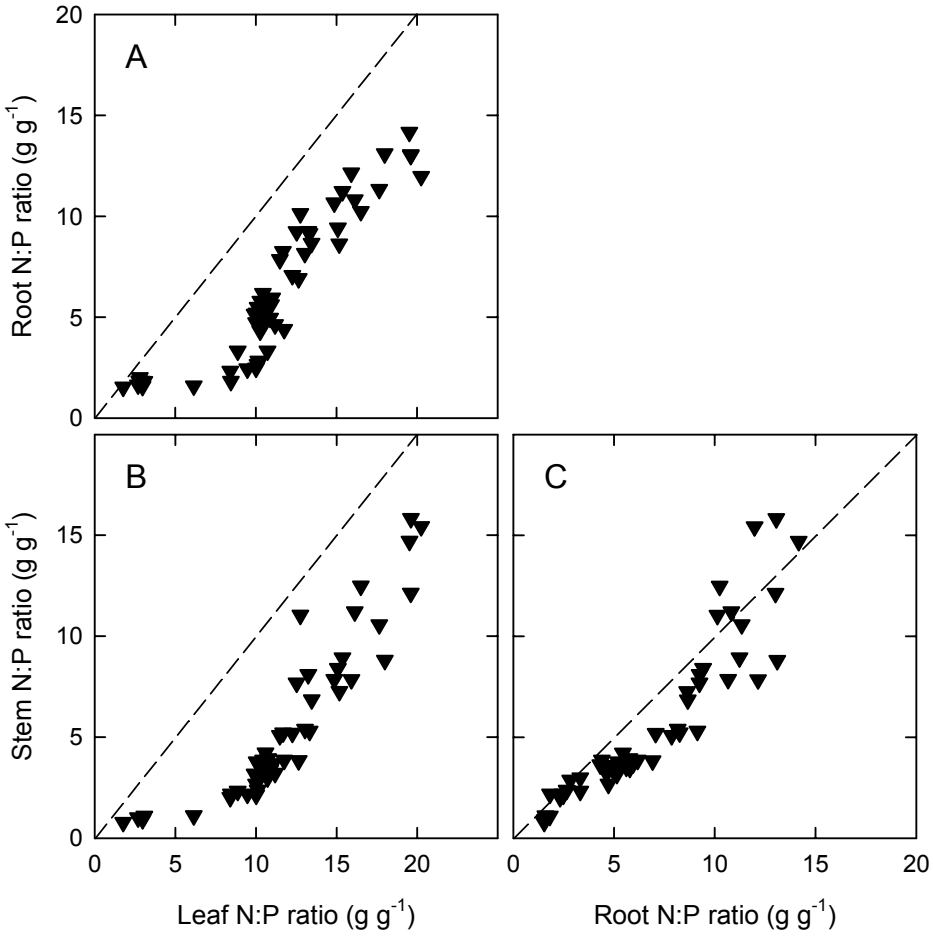


Figure 6. The nitrogen to phosphorus ratio of roots plotted against that of leaves (A), the nitrogen to phosphorus ratio of stems plotted against that of leaves (B), and the nitrogen to phosphorus ratio of stems plotted against that of roots (C) for seedlings of *Ficus insipida*. Dashed lines represent one-to-one lines.

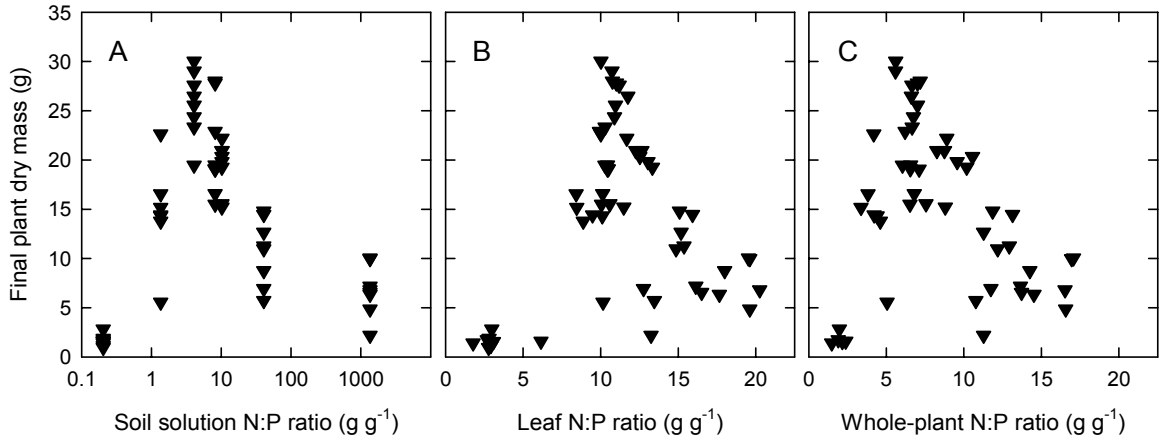


Figure 7. Final plant dry mass of *Ficus insipida* seedlings plotted as a function of the nutrient solution nitrogen to phosphorus ratio (A), leaf nitrogen to phosphorus ratio (B) and whole-plant nitrogen to phosphorus ratio (C).

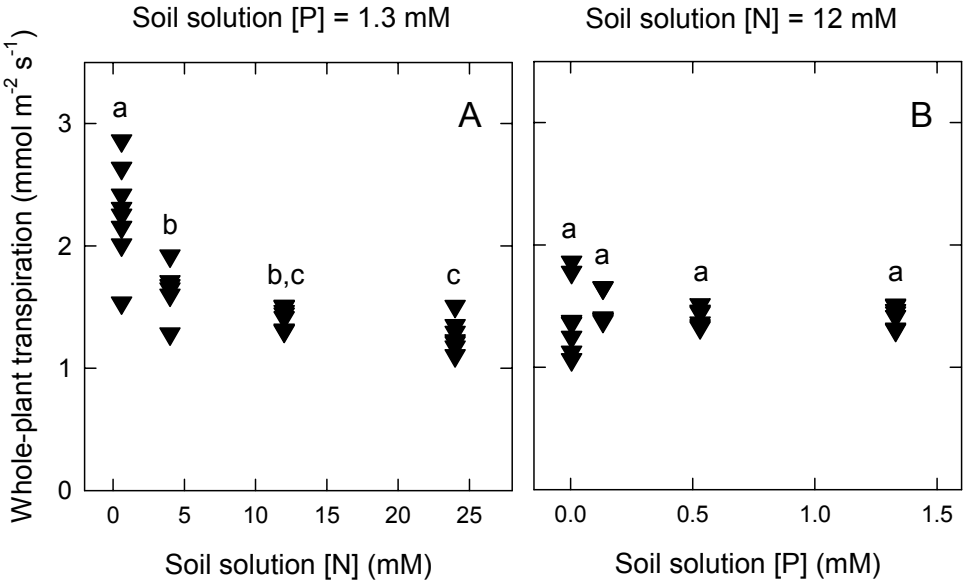


Figure 8. Daytime transpiration of *Ficus insipida* seedlings determined gravimetrically plotted against nutrient solution nitrogen concentration with phosphorus concentration held constant (A) and nutrient solution phosphorus concentration with nitrogen concentration held constant (B). Different lower-case letters within a panel indicate significantly different means at $P<0.05$.

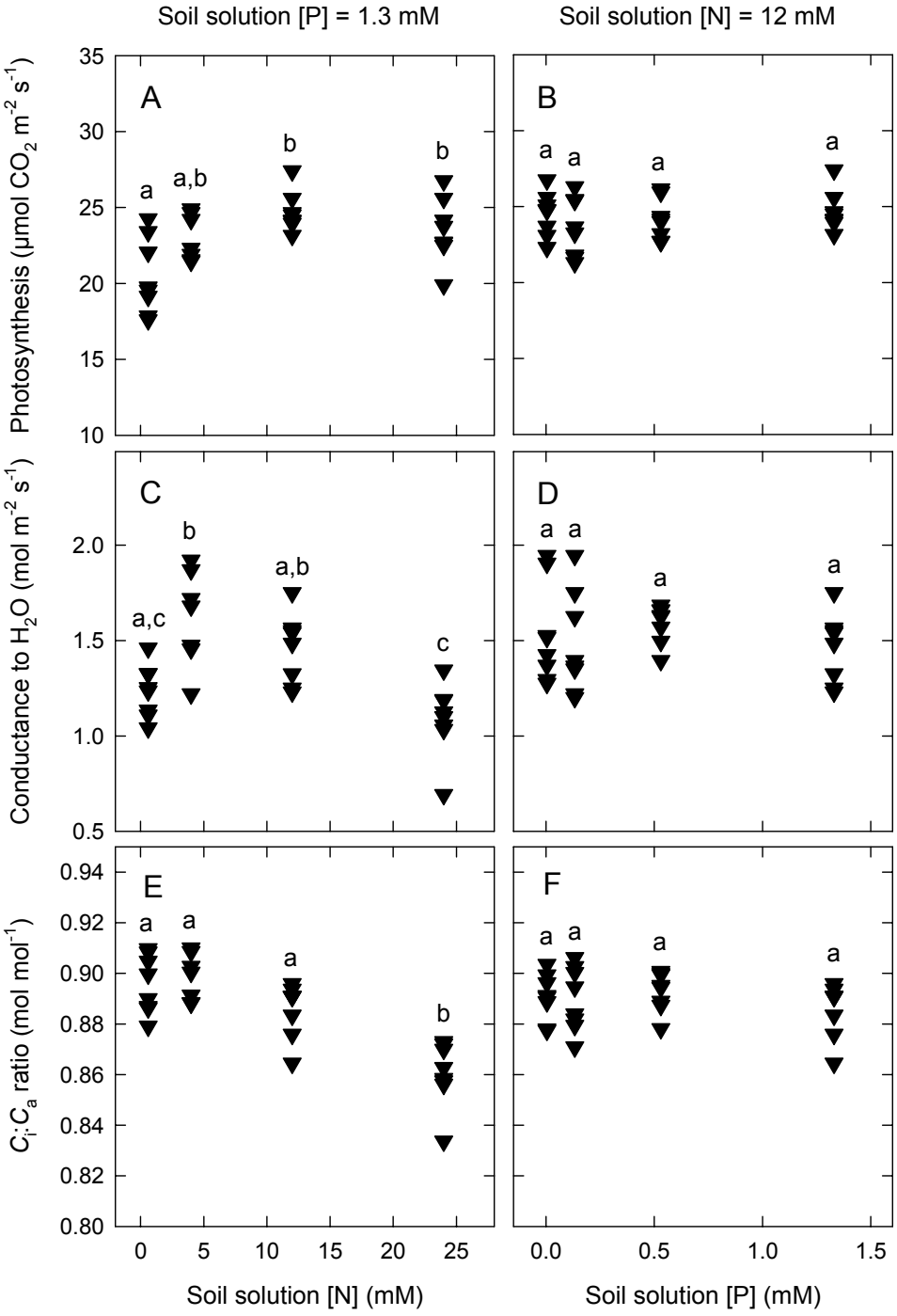


Figure 9. Photosynthesis (A and B), stomatal conductance (C and D), and the ratio of intercellular to ambient CO_2 mole fractions, c_i/c_a (E and F) plotted as functions of nutrient solution nitrogen and phosphorus concentration for seedlings of *Ficus insipida*. The left-hand panels (A, C, and E) show variation as a function of nutrient solution nitrogen concentration with nutrient solution phosphorus concentration held constant at 1.33 mM. The right-hand panels (B, D, and F) show variation as a function of nutrient solution phosphorus concentration with nutrient solution nitrogen concentration held constant at 12 mM. Different lower-case letters within a panel indicate significantly different means at $P<0.05$.

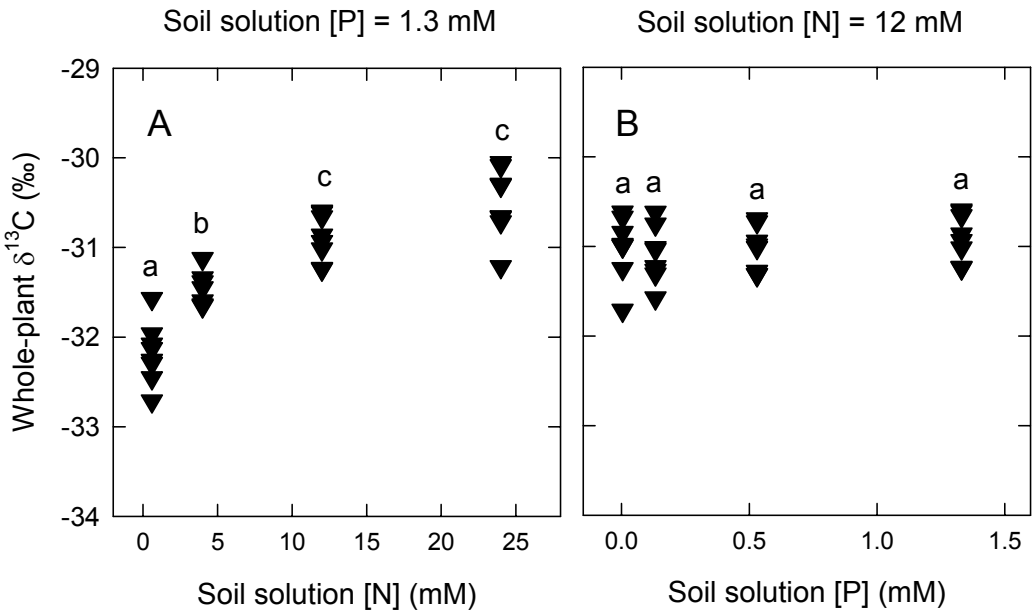


Figure 10. Whole-plant carbon isotope ratio ($\delta^{13}C$) of *Ficus insipida* seedlings plotted against nutrient solution nitrogen concentration with phosphorus concentration held constant (A) and nutrient solution phosphorus concentration with nitrogen concentration held constant (B). Different lower-case letters within a panel indicate significantly different means at $P<0.05$.

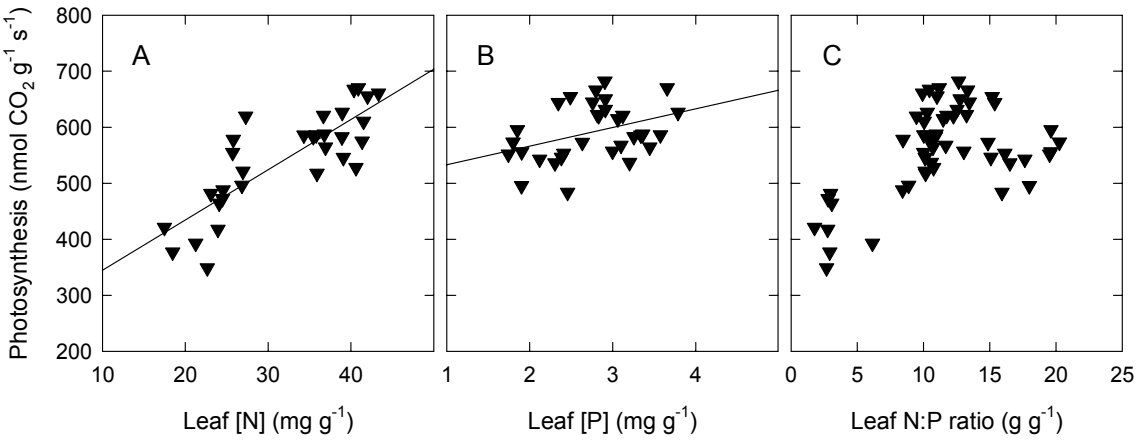
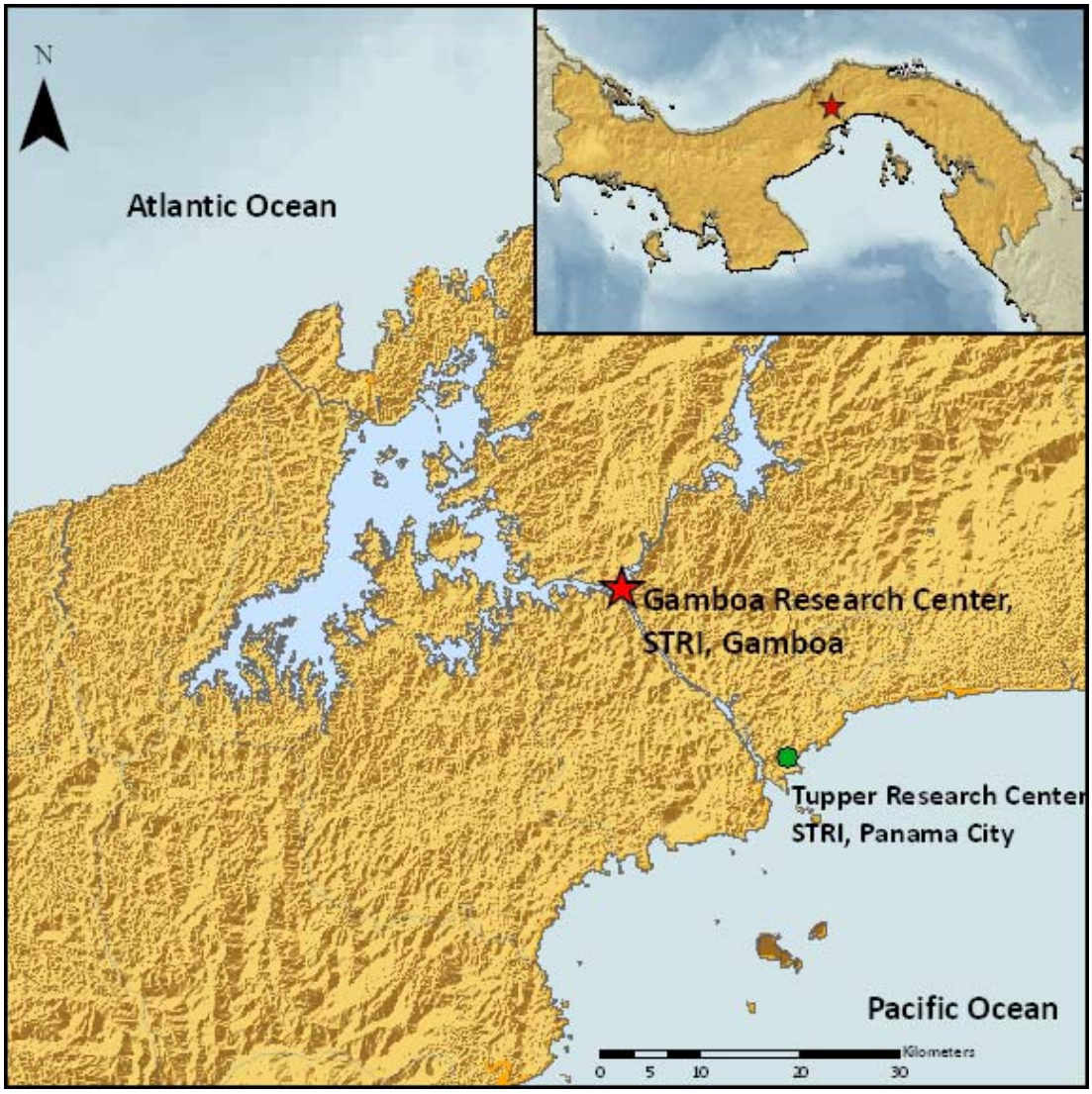


Figure 11. Mass-based photosynthesis plotted against leaf nitrogen concentration (A), leaf phosphorus concentration (B), and leaf nitrogen to phosphorus ratio (C). Data in (A) are for plants grown at variable nitrogen concentration and constant phosphorus concentration, and data in (B) are for plants grown at variable phosphorus concentration and constant nitrogen concentration. Data in (C) are for both sets of plants. Solid lines in (A) and (B) are least-squares linear regressions. Both are significant at $P<0.05$.

Maps:



Map 1. Location of laboratory facilities at the Tupper Research Center in Panama City, Panama and greenhouse facilities at the Gamboa Research Center, Gamboa, Panama