AN ABSTRACT OF THE THESIS OF

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Title: The Effect of Nitrogen and Phosphorus on Growth, Carbon Allocation and Nitrogen Fixation of Red Alder Seedlings

Abstract approved: Signature redacted for privacy.

David E. Hibbs

The effect of solution nitrogen (N) and phosphorus (P) concentrations on biomass production and N_2 fixation of red alder (Alnus rubra Bong.) seedlings grown in perlite-filled pots in a climate controlled growth room were studied. Nodulated seedlings were subjected to 12 different nutrient solution combinations of nitrogen and phosphorus and one sodium control treatment. Carbohydrate allocation, nitrogen fixation (using acetylene reduction (AR) methods) and leaf N and P concentrations were measured to determine the relative importance of external nitrogen and phosphorus concentrations on plant growth and nutrient status, nodule production, and nitrogen fixation activity.

Nodule biomass per plant declined with increasing solution N concentrations (1, 10, 100, 1000 mg N/l). Nodule AR rates remained relatively stable over most of the treatments. This study, however provides some evidence that nodule and plant AR rates can be enhanced at treatment

combinations of 100 mg N/l and 100 mg N/l over all other treatment combinations of N and P used in this study.

Leaf P concentrations increased with increasing solution P concentrations (10, 100, 1000 mg P/l). Nodule biomass per plant, nitrogenase activity (measured as nodule AR rates) and plant AR rate were greatest at 100 mg P/l. Plant biomass production was greatest at solution concentrations of 100 mg N/l combined with 10 or 100 mg P/l. All measured variables were depressed at high solution concentrations of nitrogen (1000 mg N/l) and phosphorus (1000 mg P/l) probably due to the adverse effects of high leaf nutrient concentrations.

Plant nitrogen fixation declined with increasing solution N concentration. The nitrogen content of leaves per plant, however, was greater at 100 mg N/l than when N was present at 1 and 10 mg N/l in solution. This suggests that there was a shift in the relative contribution from fixed N as a major contributor to the plant nitrogen pool at solution N concentrations of 1 and 10 mg N/l, to mineral N at solution concentrations of 100 mg N/l and 1000 mg N/l.

Increased demand for carbohydrate by nitrogen fixation at low external N concentrations (1 and 10 mg N/l) was associated with reduced plant growth. First, leaf nitrogen concentrations appeared to be optimum for plant growth in all treatments, thus the reduction in plant growth at 1 and 10 mg N/l was not due to a nitrogen deficiency.

Second, the decline in nitrogen fixation with increasing solution N concentrations would result in a reduced demand for plant carbohydrates by the nodules. At 100 mg N/l carbohydrate was directed away from the nodules to other plant parts resulting in an increase in growth. Finally, it was concluded that the effect of solution N concentrations on alder nitrogen fixation and growth appeared to be through a shift in the allocation of carbohydrate to nodules and other plant parts.

The effect of solution P concentrations on alder nitrogen fixation appeared to be through a stimulation of nodule biomass up to solution P concentrations of 100 mg P/l. Leaf P concentrations ranged from optimum to potentially toxic for plant growth. Biomass production was highest when solution P concentrations were at 10 mg P/l and leaf P concentrations were between 0.2% and 0.4%.

An interaction between N and P indicates that each may modify the effect the other has on plant growth and nitrogen fixation. Leaf nutrient concentrations, nodule biomass, and nodule AR rates were influenced by an interaction between N and P. An increase in treatment P concentrations to 100 mg P/l modified the negative effect of solution nitrogen on nodule biomass so that the rate of decline of nodule biomass was reduced compared to 10 mg P/l solution treatments. Plant AR activity increased significantly, when treated with a combination of 100 mg N/l

and 100 mg P/l in solution, as compared to all other treatments. This increase was a combined result of P enhancement of nodule biomass and a stimulation of nodule AR activity by N and P as plant growth increased and more carbohydrate was produced. It appears that nitrogen fixation may be enhanced when low amounts of external nitrogen are present and P availability is not limited.

The negative effect nitrogen concentrations on red alder nitrogen fixation have been reported in other studies. However, the importance of phosphorus to nitrogen fixation has only recently been brought under investigation. This study indicates that high P availability can modify the negative effect of external nitrogen concentrations on nitrogen fixation and increase the potential contribution of nitrogen to the system by the nitrogen fixing plant.

Effect of Solution Nitrogen and Phosphorus on Growth, Carbon Allocation and Nitrogen Fixation of Red Alder Seedlings

by

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INTRODUCTION

Red alder (Alnus rubra Bong.), an actinorhizal host plant capable of forming a symbiotic association with the nitrogen-fixing actinomycete, Frankia, can be an important contributor to the nitrogen pool of nutrient poor forest soils (Zavitkovski and Newton 1968, Tarrant 1968). The accretion of nitrogen in the soil, through decomposition of nitrogen-rich alder litter, has increased the productivity of species growing in association with alder (Miller and Murray 1979, Hansen and Dawson 1981). Mixed plantations of alder and a crop species have been considered as alternatives to fertilization (Miller and Murray 1979). However,the benefit of using a nitrogen-fixing species is often limited on sites of high fertility and when the growth potential of the crop tree is suppressed by competition with the nitrogen-fixer (Binkley 1983, Cole and Newton 1986).

Nitrogen is the nutrient most commonly limiting to plant growth in the Pacific Northwest (Date 1973). Most plants can acquire nitrogen from the soil as nitrate (NO_3^-) or ammonium (NH_4^+) (Moorby and Besford 1980). An actinorhizal plant can utilize nitrogen through two enzyme

systems. Nitrate reductase occurs in the roots and reduces soil supplied NO_3^- to ammonium for plant use. Nitrogenase, found only in N_2-fixing organisms, occurs in the root nodules and reduces atmospheric nitrogen obtained through the fixation process (Quebedeaux 1979, Schubert 1986). If a soil medium is low or deficient in N, an actinorhizal plant may obtain all or part of the nitrogen required for growth through fixation of atmospheric nitrogen (Leaf et al. 1958, Zavitkovski and Newton 1968).

Photosynthesis and nitrogen fixation

Photosynthesis is the primary physiological process providing energy to support atmospheric N_2 fixation (Hardy and Havelka 1976, Quebedeaux 1979). Carbohydrates produced during photosynthesis are allocated to various parts of the plant, including the nodules, and provide energy for the fixation process (Stewart 1982, Gordon et al. 1985, Kouchi et al. 1986). Plant nitrogen fixation, in turn, provides the nitrogen for increased photosynthesis and carbohydrate production (Figure 1). Although the total carbohydrate stored in nodules is a very small amount of total plant biomass, the flux of carbohydrate to the nodules is large and can represent up to one quarter of the total photoassimilate production in leaves (Kouchi et al. 1986).

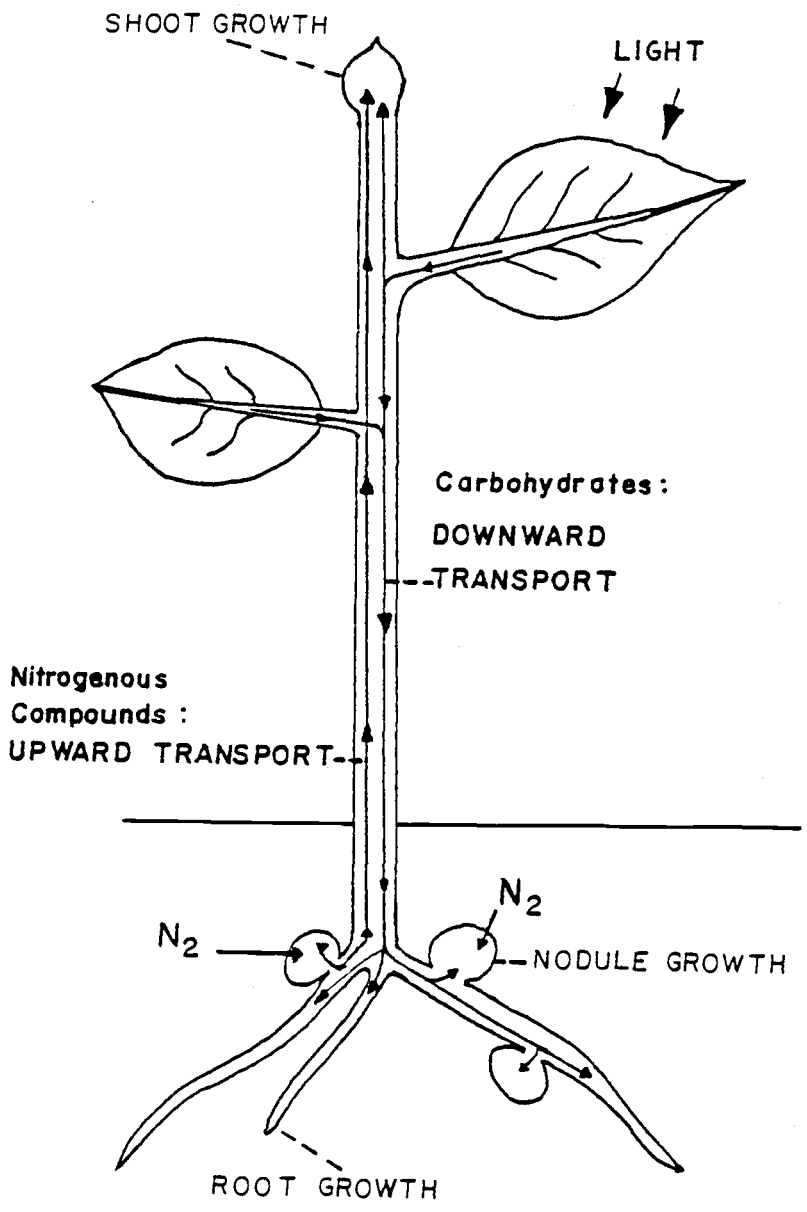

Figure 1: Generalized whole plant carbon and nitrogen transport system of red alder.

Generally, those factors which influence photosynthesis also influence nodule and plant nitrogen fixation (Quebedeaux 1979). Changing photosynthetic rates, as regulated by diurnal changes in light intensity, also change the activity of the enzyme nitrogenase in Alnus glutinosa (L.) Gaertn. (black alder) (Wheeler and Lawrie 1986). Field and greenhouse studies (Wheeler 1971, Dawson and Gordon 1979) of nitrogen fixation in Alnus glutinosa indicate that nitrogenase activity is highest at midday when light intensity is the greatest. Also, an increase in photosynthesis, stimulated by an increase in light intensity, was directly correlated with increased nodule AR rate (nitrogenase activity) and increased nodule biomass over a longer term in soybean (Glycine spp.) (Hardy and Havelka 1976, Bethlenfalvay and Phillips 1978) and in Alnus glutinosa (Gordon and Wheeler 1978). Nitrogenase activity can be reduced by overtopping and shading of red alder in mixed stands (Hielman and Stettler 1983) and competition for light in monocultures (Bormann 1981, Smith 1983). Alnus incana (L.) Moench. seedlings subjected to defoliation or absence of light have decreased nitrogenase activity almost immediately upon commencement of treatment (Huss-Danell and Sellstedt 1983). The importance of the interrelationships between current photosynthate production and nitrogen fixation are emphasized by these studies.

Effects of external N on nitrogen fixation

A depression in nitrogen fixation has been found to occur with increasing external nitrogen concentrations for both legume and non-legume nitrogen-fixing plants (Stewart 1982, Tjepkema and Schwinter 1986). The inhibition of nodulation in legumes by external solution nitrogen has been known for many years but recently it has been shown that solution nitrogen also inhibits nitrogenase activity of the nodules (Gibson and Pagan 1977, Streeter 1981).

Nutritional studies of leguminous and non-leguminous host plants found that plant requirement for fixed nitrogen may change with the availability of external nitrogen (Ingestad 1980). Atmospheric fixation of nitrogen and nodulation declines in Alnus when the concentration of nitrogen in the soil and in solution increases (Zavitkovski and Newton 1968, Nordmeyer 1969, Benecke 1970, Bond et al. 1954, Ingestad 1980). At low external nitrogen concentrations nitrogenase activity of the nodules can be enhanced in alder (Bond et al. 1954) and in legumes such as soybean (Streeter 1985, Imsande 1986). Plant nitrogen fixation, nodule AR rates, and nodule biomass were enhanced up to an external solution concentration of 28 mg N/l, after which nodule biomass and nodule AR rates declined in pea (Pisum spp.) (Bethlenfalvay et al. 1978). Bethlenfalvay et al.

(1978) suggested that the uptake of N from low solution concentrations improves biomass production and allocation of carbohydrate to the nodules in young seedlings.

There have been indications that nodule biomass and nitrogenase activity have varying responses to different external nitrogen concentrations. In a study of the effect of external N on nitrogen fixation in soybean, Imsande (1985) found that below 28 mg N/l nodule production was inhibited and above 84 mg N/l nitrogenase activity was inhibited indicating that the effect of external nitrogen on nitrogen fixation may be a function of external N concentration.

External concentrations of combined nitrogen (NO_3^- and NH_4^+) in solution below 20 mg/l have enhanced plant nitrogen fixation through increased nodule development, although nodule AR rates were decreased in Alnus rubra (Monaco et al. 1981). Nodule dry weight was increased when red alder was grown in soil N concentrations up to 30 mg/l of nitrate, however, further increases in N resulted in decreased nodule biomass (Zavitovski and Newton 1968). Nodule development of Alnus glutinosa was suppressed at 5 mg N/l (Quispel 1954), between 10 and 50 mg N/l (MacConnell and Bond 1957), and only when more than 100 mg N/l was present in solution (Bond et al. 1954).

The lack of specific agreement among investigators on the degree to which external N concentrations affect

nodule biomass and nitrogen fixation activity may be related to differences in the conditions under which each experiment was performed, such as the degree of control of solution pH, the number of complete changes of nutrient solution, the environmental conditions under which the plants grew, and the genetic differences of species. Nitrogen fixation is known to be sensitive to changes in temperature, moisture, pH, and the nutrient status of the environment (Wheeler and McLaughlin 1979).

Cultured Frankia is also affected by solution N concentrations. Vesicles were formed at solution concentrations of 52 mg N/l but lacked the nitrogenase enzyme that was prominent at 0 mg N/l (Meesters et al. 1985), which suggested that the biosynthesis of the nitrogenase enzyme was inhibited by solution N. Tjepkema and Winship (1980) found that vesicle formation of cultured Frankia was completely suppressed at 14 mg N/l in solution. The effect of solution N on Frankia is expected to be modified in symbiosis with its host plant.

Although there is general agreement that increasing external nitrogen concentrations decrease N_2 fixation, either through changes in the nitrogenase activity (often measured as acetylene reduction activity of the nodules) or through reduced nodule development, the mechanism by which nitrogenous compounds might suppress nodule formation and activity is largely unknown. External nitrogen

may influence plant factors that control nitrogen fixation, such as the concentration and saturation of the enzyme nitrogenase, the amount of photosynthate transported to the nodules, and the strength of other plant sinks of carbohydrate (Hardy and Havelka 1976, Quebedeaux 1979).

Nodule development is thought to be inhibited by a shift of carbohydrate demand from nodules to other energy demanding processes, such as nitrate reduction in the leaves (Noel et al. 1982). Also, an inhibition of nodule function or nitrogenase activity, related to the accumulation of nitrogen products, can lead to a decrease in carbohydrate demand of nodules (Gibson and Pagan 1977, Streeter 1985) and therefore reduced nodule development. Also, as the internal concentration of nitrogen increases in legumes, changes in the activity of different enzymes are known to occur (Smith et al. 1985, Silsbury et al. 1986). Glutamine, a plant amino acid present in both legumes and non-legumes, has been found to increase in concentration with increasing N assimilation and is known to directly inhibit nitrogenase biosynthesis and vesicle formation by cultured Frankia (Hayes et al. 1985).

Effects of external phosphorus on nitrogen fixation

A few studies suggest that phosphorus is important for nitrogen fixation. A phosphorus deficiency can

affect the rate of photosynthesis and carbon partitioning though limitation of ATP synthesis and ribulose-1,5 bisphosphate carboxylase activity (Dietz and Foyer 1986). Also, the importance of phosphorus in the transport of reduced CO_2 from the chloroplasts to the cytoplasm (Heldt et al. 1977, Huber et al. 1985, Robinson and Baysdorfer 1985) for distribution among various plant sinks is well known. Israel (1985) concluded that the P status of a plant has a specific role in both plant growth and nitrogenase activity. Improvement of P status increased whole plant dry weight and nitrogen accumulation, total leaf area, nodule weight and acetylene reduction activity of the nodules in soybean.

An increase in the supply of solution phosphorus has been shown to increase nitrogen fixation in legumes through stimulation of nodule AR activity and formation (Gates and Wilson 1974, Smith and Daft 1977, Hart et al. 1981a, 1981b, Israel 1985). Solution concentrations from 50 mg P/l to 500 mg P/l have increased plant growth, nodule biomass and plant fixation activity in clover (Trifolium spp.) and lotus (Lotus spp.) (Hart et al. 1981a). When comparing the response to P of N_2-fixing plants and plants supplied with solution N, Hart et al. (1981a) found that clover and lotus had similar growth responses to increased P supply when external N was also supplied but responded very differently to P when fixed N was the

only N source. They concluded that the response to P of N_2 fixation depended on each species tolerance to P deficiency when in symbiosis with a N_2-fixing endophyte. These results also suggest that plant requirement for P may be greater when relying on nitrogen fixation for its nitrogen supply than when mineral N is available. The mechanism by which P influences nodule biomass and nitrogenase activity is unknown. Nodule dry weight and nodule AR activity have responded to the addition of P before stem growth responses could be observed in pea (Jakobsen 1985). From these observations it was inferred that P directly affected the nodules during the first 20 days after emergence from seed. Robson et al. (1981) found that, after 50 days of growth and treatment, that an increase in nodule weight and plant AR activity in clover paralleled an increase in plant biomass as P supply increased. Nitrogenase activity, however, remained relatively stable over the experimental period and with increasing P supply. Generally, these findings point to the improvement of nitrogen fixation by increased P supply through the increased development of nodules. Although there is some controversy about the effect of P on nodule fixation activity, there is general agreement that the effect of P on nodule biomass is a positive one.

Studies on the effects of phosphorus on Alnus spp. have found that increased P uptake enhances the number of nodules in Alnus rubra (Zavitkovski and Newton 1968), Alnus viridis (Nordmeyer 1969) and Alnus glutinosa (Seiler and McCormick 1982), and, therefore, increased nitrogen fixation on a plant basis.

A study of the interaction of nitrogen and phosphorus on the legume, Stylosanthes humilis revealed that phosphorus can ameliorate the depressing effect of solution nitrogen (5.7 to 60 mg N/l) on nodule biomass but only at P concentrations greater than 125 mg P/l. Below this level of P, all solution nitrogen concentrations had a negative effect on nodule biomass (Gates and Wilson 1974). Plant growth has also been enhanced as N_2 fixation supplied the plant with more fixed N as P supply increased than when P was deficient (Hart et al. 1986). There have been no studies on the interaction of N and P on growth and nitrogen fixation in red alder.

Nutrient status

There have been few studies which have examined the nutrient requirements of red alder (Hughes et al. 1968, Russell et al. 1968) and of other Alnus spp. (Ingestad 1980, Prégent and Camiré 1985). Generally, nutrient deficiencies in red alder are identified by reductions in

growth relative to the growth of non-nutrient limited plants. Correlations are made between the nutrient concentrations of leaves and reductions in plant growth. Growth reductions, however, have been noted over a wide range of leaf nutrient concentrations so that evaluating a nutrient deficiency by leaf nutrient concentrations must be done with caution (Hughes et al. 1968, Ingestad 1980).

Foliar analysis of red alder indicates that leaf N and P concentrations less than 2.4% and 0.16% respectively, can result in a depression in plant growth (Hughes et al. 1968). Nutrient analysis on red alder leaves grown in the field indicate that leaf concentrations of 3.05% N and 0.23% P are not uncommon (Binkley 1983). When the solution nitrogen concentration was low leaf N concentrations ranged from 2.4% to 3.4% in Alnus incana (Sellstedt et al. 1983). Leaf P concentrations below 0.13% are thought to be deficient for the growth of Alnus glutinosa and Alnus crispa (Prégent and Camiré 1985). Most values reported for alders generally represent a non-deficiency plant nutrient status, however, Ingestad (1980) found that Alnus incana seedlings relying on N_2 fixation as the only source of nitrogen had optimal leaf N concentrations of 2.5% but only half the relative growth rates and biomass production of plants supplied with an external N source.

Increased leaf N concentrations can increase net

photosynthesis and carbon metabolism in soybean (Robinson and Baysdorfer 1985). Stimulation of these processes by N leads to greater photoassimilate production and transfer and a possible increase in nodule and plant nitrogen fixation activity. The activity of ribulose-1,5-bisphosphate and other enzymes involved in the Calvin cycle and in the transport of carbon assimilates from the chloroplasts to leaf cytoplasm are stimulated by increased leaf nitrogen concentrations (Robinson and Baysdorfer 1985).

Increased leaf P concentration can be accompanied by an increase in N concentration in leaf or shoot tissue in clover (McLachlan and Norman 1961, Robson et al. 1981) and pea (Jakobsen 1985) relying on fixed nitrogen as a source of nitrogen. However, when given an external source of N, P had a negative effect on shoot tissue N concentrations and growth in nodulated peas (Jakobsen 1985). A 10 times increase in solution P and 3 times increase in leaf P concentration (0.09% to 0.28%) was correlated with increased leaf nitrogen concentrations from 1.72% to 2.43% in Alnus glutinosa not supplied with an external N source (Prégent and Camiré 1985). These results suggest that P can enhance nodule AR activity and therefore increase N availablity to the plant. Also, improved plant nitrogen status in plants utilizing fixed N and supplied with P has been correlated with improved plant growth in clover (McLachlan and Norman 1961, Robson et al. 1981).

Carbohydrate production and nitrogen fixation

The reduction, assimilation, and transport of either atmospheric nitrogen through symbiotic nitrogen fixation or mineral nitrogen from the soil requires plant energy expenditures in the form of carbohydrates. Carbohydrates produced by the host that are transported to the nodules and used in the nitrogen fixation process can represent a loss of potential biomass production and growth for the host plant (Winter and Burris 1968, Paul and Kucy 1981, Schubert 1982, Ryle et al. 1984, Sellstedt and Huss-Danell 1986). A 19% increase in biomass yield might occur if nodules were not present and the nitrogen required for the growth of Alnus could be acquired without energy cost (Tjepkema 1985). The energy requirement of N_2 fixation in Alnus is thought to be comparable to that of legumes, where as much as 18.8 g of plant carbohydrate needed for each gram of nitrogen fixed (Gutschick 1978, Tjepkema and Winship 1980, Tjepkema 1985).

Comparative measures of biomass production between nodulated legumes fixing N and non-nodulated legumes supplied with N have shown that carbohydrate demand may be greater (Pate et al. 1979, Fincke et al. 1982, Marcus-Wyner and Rains 1983), or the same (Minchin and Pate 1973) or lower (Lambers et al. 1980) for meeting nitrogen requirements through N_2 fixation compared to the carbohydrate

requirement of nitrate reduction. The nutrient conditions, however, had differed for the nodulated and non-nodulated seedlings in these studies so that the results are not directly comparable. Studies of Alnus spp. have indicated that the fixation process requires greater plant energy expenditures than the energy required for nitrate reduction as a result of the extra respiratory load and carbohydrate demand of nodule development and maintenance (Huss-Danell et al. 1982, Akkermans et al. 1983, Lopez et al. 1986).

While biomass production has been found to be lower for some nitrogen-fixing legumes than non-nodulated legumes supplied with mineral nitrogen due to the high carbohydrate costs of the N_2 fixation system (Bethlenfalvay et al. 1978, Marcus-Wyner and Rains 1983), nodulated Alnus incana seedlings supplied with low concentrations of mineral N had higher relative growth rates and biomass production than non-nodulated seedlings supplied with the same N source (Ingestad 1980). However, at high N concentrations the highest maximum growth rates were exhibited by the non-nodulated seedlings. Alternately, Benecke (1970) found that nodulated Alnus glutinosa seedlings produced greater biomass when grown without an external nitrogen source than non-nodulated alders given solution N. Sellstedt (1986) suspects that the non-nodulated alders may not have take up all the available nitrogen in solution and, therefore, had a lower nitrogen status

than nodulated plants. Huss-Danell and Sellstedt (1985) and Sellstedt (1986) could not find differences in total biomass production of nodulated Alnus incana seedlings and non-nodulated alders supplied with mineral N at the same rate as the nodulated alders were fixing N.

Summary

Studies of the effect of mineral nutrients such as N and P on nitrogen fixation in legumes have been intensive, particularly in crop systems where nitrogen-fixing plants are a potential source of N fertilizer (Stewart 1982). Investigators have tried to establish whether a specific nutrient such as nitrogen or phosphorus acts directly upon the nodules or indirectly via the host plant to alter nitrogen fixation (Sprent and Minchin 1983).

Increasing external nitrogen concentrations tends to decrease nodule N_2-fixing activity and nodule production (Streeter 1985). Nodule biomass production is often inhibited when external nitrogen concentrations reach 28 mg N/l. Nitrogenase activity of red alder has been reported to be inhibited by external solution concentrations of 20 mg N/l (Monaco et al. 1981). External solution P concentrations to stimulate biomass production and nodule AR activity (Hart et al. 1981b), through an alleviation of a phosphorus deficiency. The range of solution P

concentrations in which a stimulation of nitrogen fixation has been noted, is fairly wide (50-500 mg P/l). The mechanisms by which external concentrations of N and P influence nitrogen fixation are poorly understood. External N is thought to inhibit nodule development by shifting the allocation of carbohydrate away from the nodules to other plant parts. As mineral N is taken up by the plant, other energy demanding processes develop, such as nitrate reduction. Nitrogenase activity is thought to be inhibited by increasing plant nitrogen concentrations as nitrogen uptake increases with the increasing availability of solution nitrogen.

Increasing P availability is thought to stimulate nitrogen fixation through increased nodule development and increased nitrogenase activity. There has been some controversy as to the mechanism by which P stimulates nitrogen fixation. Robson et al. 1981) have correlated an increase in plant vigor with increasing nitrogen fixation as P supply increases. Hart et al. (1981a) did not find a correlation between plant vigor and nitrogen fixation as P supply increased and suggested that the symbiosis was stimulated by increasing P supply.

Objectives

Few studies have examined the effects and interactions of N and P on red alder nitrogen fixation. There is a

lack of information on the effect of P on red alder growth and nitrogen fixation. The goal of my study was to investigate the effect of external nitrogen and phosphorus on growth, carbohydrate allocation and nitrogen fixation in young red alder seedlings. The specific objectives of my study were: a) to determine the response of nodule biomass production and nodule fixation rates to increasing concentrations of external nitrogen and phosphorus, b) to determine the relative importance of carbohydrate allocation to nitrogen fixation and plant biomass production and c) to determine the relative importance of nitrogen and phosphorus to red alder seedling growth.

MATERIALS AND METHODS

Four-month-old red alder seedlings were selected from a coastal clear-cut on the Cascade Head Experimental Forest, Oregon on September 1, 1986. Seedlings selected had visible nodulation and had shoot lengths of six centimeters. The roots were rinsed free of soil. Two to four seedlings were transplanted into 15 litre pots filled with 100% perlite and placed in the Forest Research Laboratory in Corvallis, OR.

Pots were connected to containers of nutrient solution by a hose system though which nutrient solutions were forced with pressure every two to three hours. A half-strength Hoaglands solution (Hoagland and Arnon 1938) was used as the base nutrient solution. Solutions containing the appropriate treatment concentrations of nitrogen and phosphorus were made up separately from the base solution and added to the containers with the base solutions to create final complete treatment solution. The composition of nutrient solutions are presented in Appendix I. Nitrogen was applied in a constant ratio of 65% NO^-_3/35 % NH^+_4 as suggested by Ingestad (1971). Sodium hydroxide was used to keep the pH of the solutions at pH 5.6 (Wheeler et al. 1981).

A 4X3 factorial treatment design used. The treatments chosen were four solution concentrations of nitrogen (1,

10, 100, 1000 mg N/l) and three of phosphorus (10, 100, 1000 mg P/l) in a completely randomized design. There were four subsamples (pots) and three to four plants per subsample in each treatment. The treatments were replicated twice. A sodium control was included as a treatment because the high P treatments contained high sodium concentrations. The sodium control contained 1000 mg N/l, 10 mg P/l, and 1000 mg Na/l and represented the concentration of Na found in the 1000 mg N/l and 1000 mg P/l.

The growth room light and temperature regimes were as follows: 16 hours of daylight at 22°C. and 8 hours of darkness at 18° C. The maximum light intensity was 150 $mE/m^2/sec$ of photosynthetically active radiation. The relative humidity in the growth room was 70%. A timer regulated the watering of pots so at least five times a day the pots were bathed with air forced nutrient solutions from below. The nutrient solution in each container was changed every two weeks until the seedlings began new leaf growth. The solutions were then changed once a week for the remaining 7 weeks. The experiment was terminated on November 21, 11 weeks after initial transplanting.

The biomass of leaves, stems, roots, and nodules were measured on the seedlings after 11 weeks of growth. Plant parts were grouped among plants within a pot, oven dried at 70°C. for 48 hours and weighed. The average weight of a plant in each pot was determined. Plant leaf

area was measured with a Li-Cor 3100 area meter. Leaf nutrient concentrations were used as indicators of plant nutrient deficiencies. Leaf nitrogen and phosphorus concentrations were measured on randomly selected leaf samples from two pots in each replication. Plant material was ground in a Wiley mill and dried for 1 hour at 70°C. Leaf material was weighed to 0.3 g for a standard Kjeldahl analysis.

Acetylene reduction assay

Acetylene reduction (Hardy et al. 1971) was used as an indicator of nodule N_2 fixation activity (total nitrogenase activity) and plant nitrogen fixation. Acetylene reduction rates were measured using a selection of nodules and root of each plant within a pot. All plants were sampled. Intact nodules with roots were placed in 56 ml bottles and capped with an air tight seal. A volume of air was removed (5.6 ml) from the capped bottles using a needle inserted through a rubber septum and replaced with 5.6 ml acetylene gas. The bottles were left in the growth room at normal ambient temperature for a one hour incubation period. A gas sample was removed from each of the bottles and transferred to a 6 ml vacutainer tube. From this tube, a 0.1 ml gas sample was removed, injected into a gas chromatograph (Hewlett Packard Model 5880) with a flame

ionization detector. The percentage of acetylene and ethylene in the gas sample were estimated from chromatograms.

Molar amounts of acetylene reduced were calculated using an equation that is a modification of the ideal gas law:

$$C_2H_4 \text{ reduced} = \frac{(\text{Volume of } C_2H_2 \text{ used}) * (\%C_2H_4 \text{ produced}) * 10000}{((273 + {}^{o}\text{C.}) * \text{nodule weight} * \text{gas constant})}$$

where the volume of acetylene injected, the proportion of ethylene produced, the kelvin temperature, the gas constant, and the weight of nodules were used to determine the nodule reduction activity.

Nodule samples used for determination of acetylene reduction rates were removed from the roots and dried for two days at 70°C. to determine nodule biomass.

Data analysis

Analyses of variance were performed using SAS software (SAS Institute Inc., 1985). Fisher's protected least significant difference (Peterson 1985) was used for testing hypotheses of treatment mean differences. Correlation analysis was performed on all variables measured. Multiple regression analyses were not reported.

RESULTS

Biomass

The F-values of an analysis of variance procedure, on the dependent variables, are presented in Table 1. All biomass variables were significant for the independent variables N and P. The interaction terms of N and P were significant for nodule biomass, plant leaf area, and shoot:root ratio (Table 1).

Total plant biomass of red alder seedlings did not differ between the treatment 1 and 10 mg N/l treatments. As solution N concentrations increased from 10 to 100 mg N/l there was a significant ($P < 0.05$) increase in plant biomass (Figure 2, Appendix II). Increasing solution P concentrations generally depressed biomass production, though the 10 mg P/l and 100 mg P/l treatments over each level of N were not significantly different. Biomass was significantly ($P < 0.05$) depressed by the 1000 mg N/l and 1000 mg P/l treatments.

Leaf, stem, and root dry matter and total leaf area, were affected similarly by N and P (Figures 3,4,5 and 6, Appendix II). A significant increase ($P < 0.05$) in biomass occurred at 100 mg N/l over biomass production other N treatment levels.

Nodule biomass declined with increasing solution N concentrations (Figure 7). The 10 and 100 mg P/l

Table 1: Analysis of variance table presenting the F-values for the dependent variables measured in this study.
* = P < 0.05, ** = P < .01, *** = P < 0.001.

Dependent Variable	Degrees of freedom	F-value N	F-value P	F-value N*P
Total biomass (g)	7	58.08***	10.81***	1.57
Leaf wt. (g)	7	81.31***	19.93***	1.97
Stem wt. (g)	7	38.40***	3.83*	1.54
Root wt. (g)	7	27.03***	5.27*	1.37
Nodule wt. (g)	7	191.68***	32.63***	8.82***
Shoot:Root ratio	7	36.56***	17.79***	4.13*
Plant leaf area (cm^2)	7	114.08***	27.18***	4.46*
Nodule fixation rate (umoles C2H2/gr/hr)	7	32.76***	10.83***	4.80**
Plant fixation rate (umoles C2H2/plant/hr)	7	65.52***	32.87***	7.52**
Leaf N (%)	3	71.32***	7.96**	2.51
Leaf P (%)	3	1.99	32.70***	0.67
Leaf N content (g)	3	45.43***	14.71***	0.68
Leaf P content (g)	3	53.53***	46.70***	10.96***
Leaf N:Leaf P ratio	3	10.89***	112.36***	3.49*

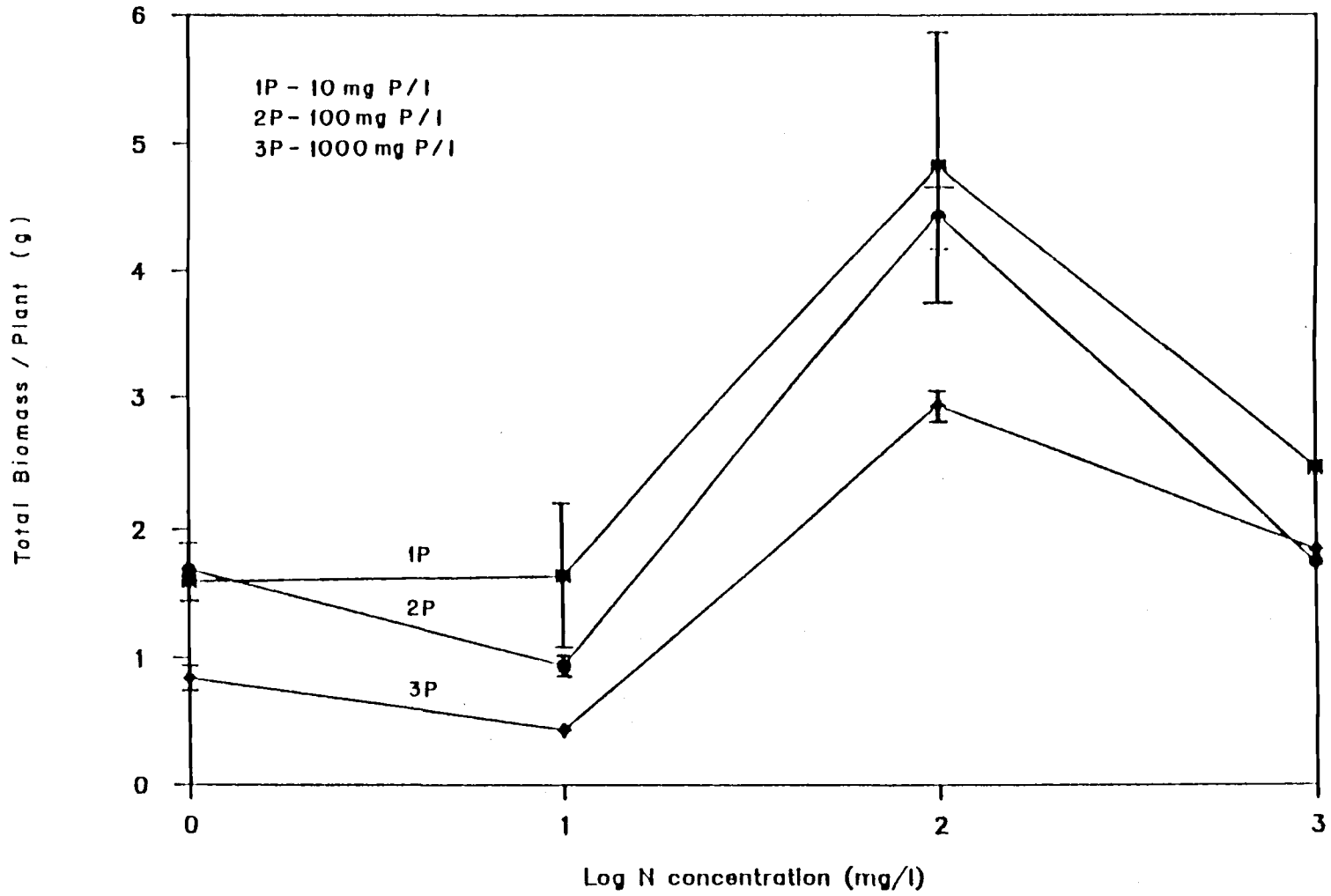

Figure 2: Total seedling biomass after 11 weeks of treatment. Means and 95% confidence limits are presented. Some confidence limits are smaller than the symbols.

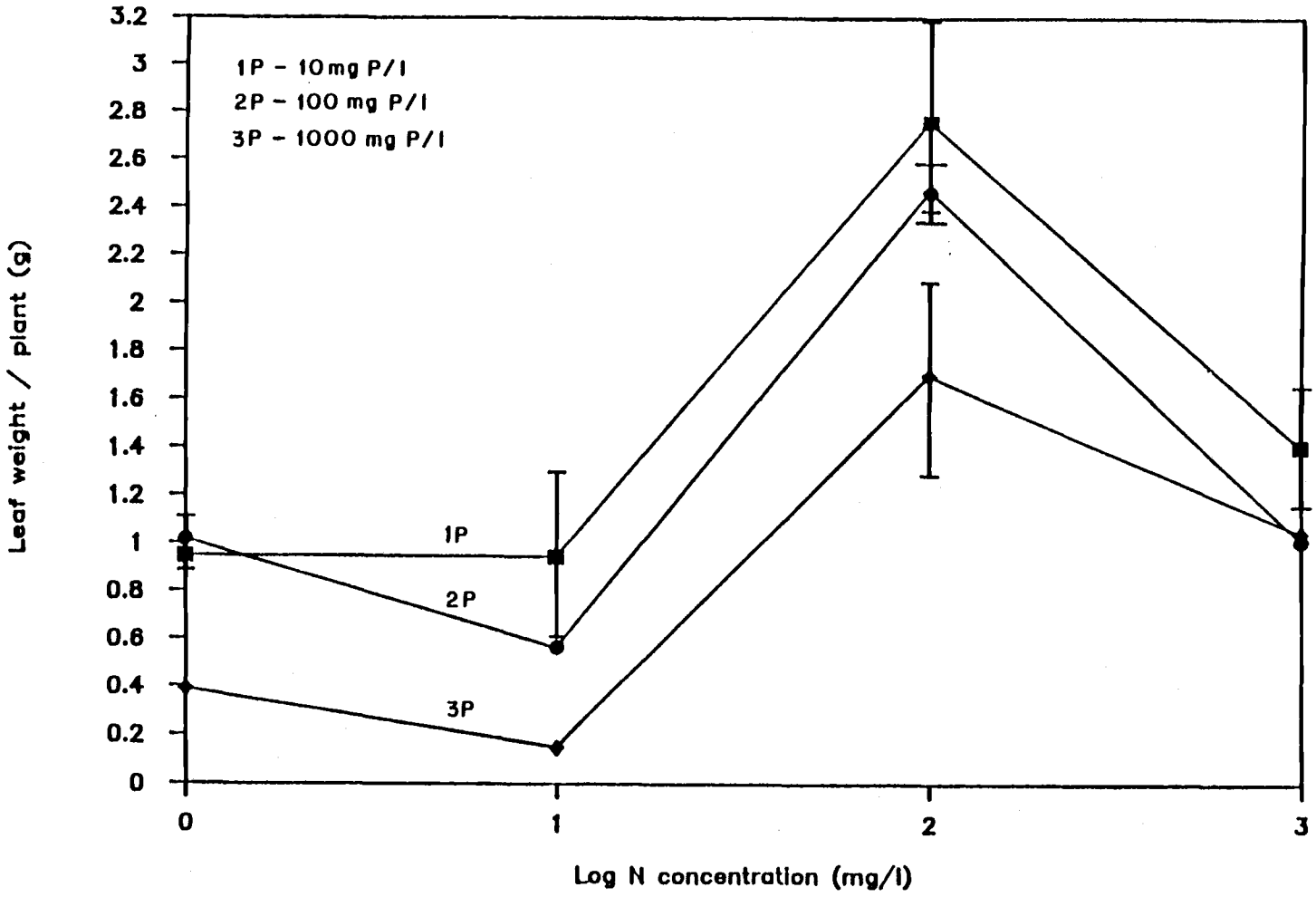

Figure 3: Leaf weight per seedling. Means and 95% confidence limits are presented. Some confidence limits are smaller than the symbols.

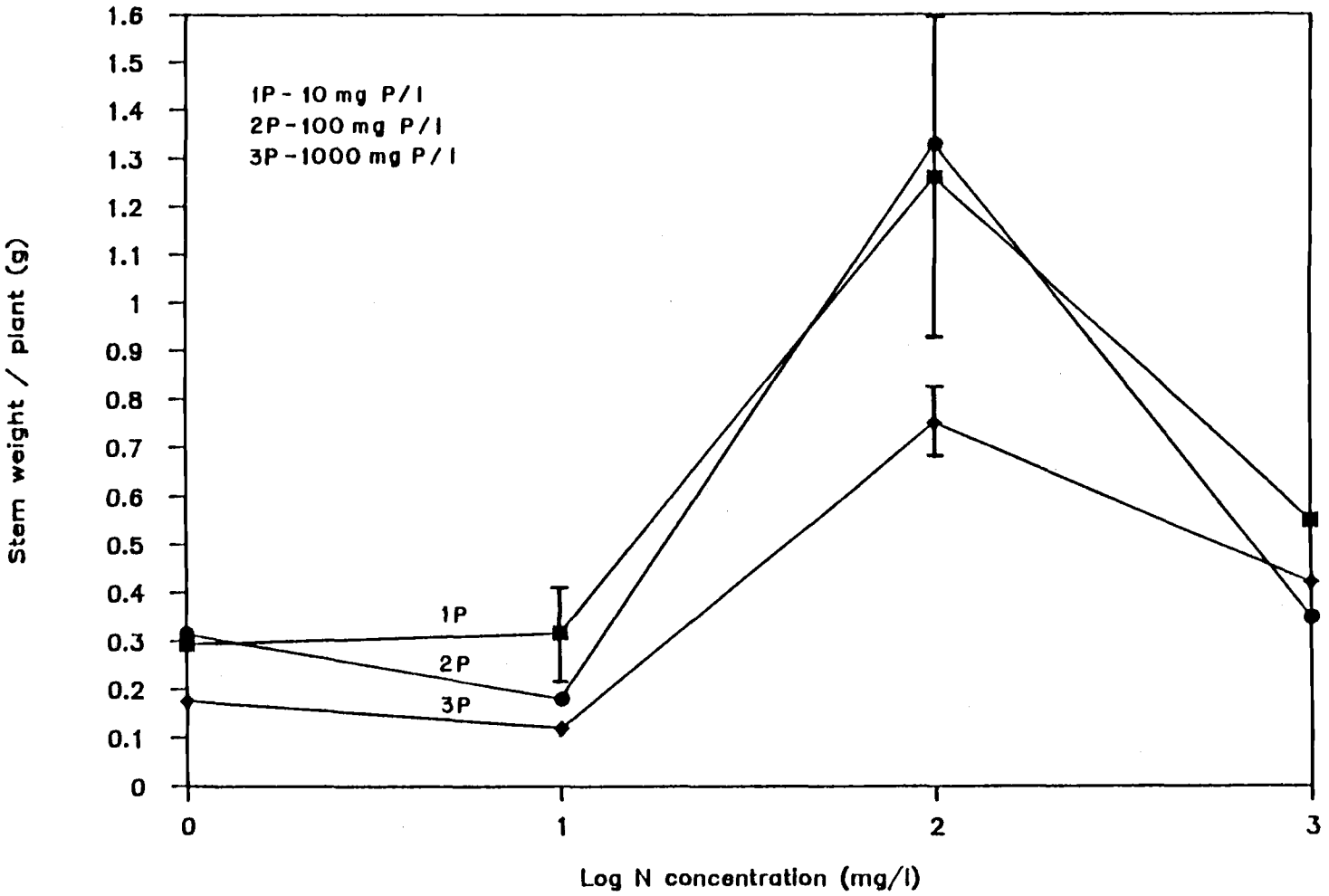

Figure 4: Stem weight per seedling. Means and 95% confidence limits are presented. Some confidence limits are smaller than the symbols.

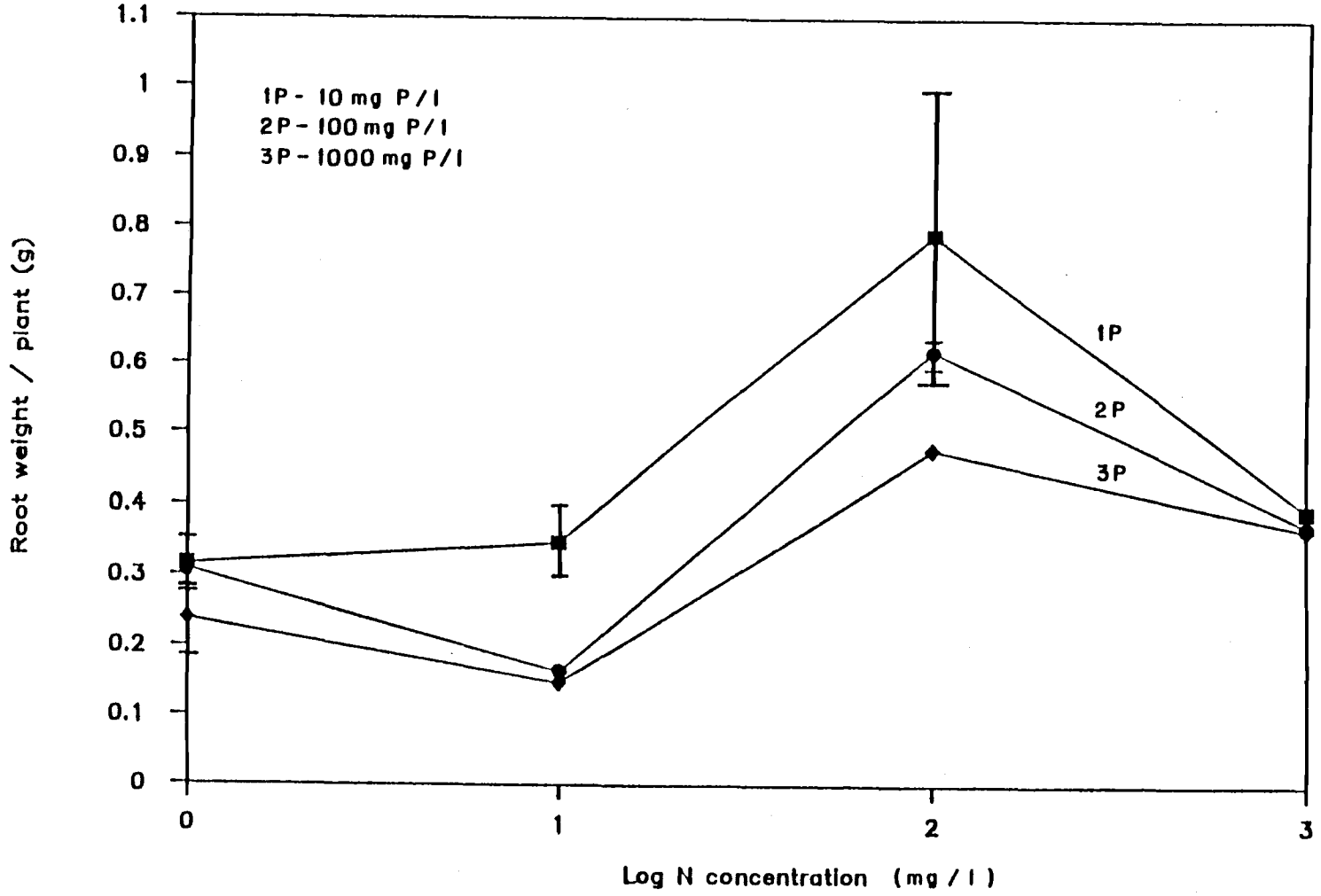

Figure 5: Root weight per seedling. Means and 95% confidence limits are presented. Some confidence limits are smaller than the symbols.

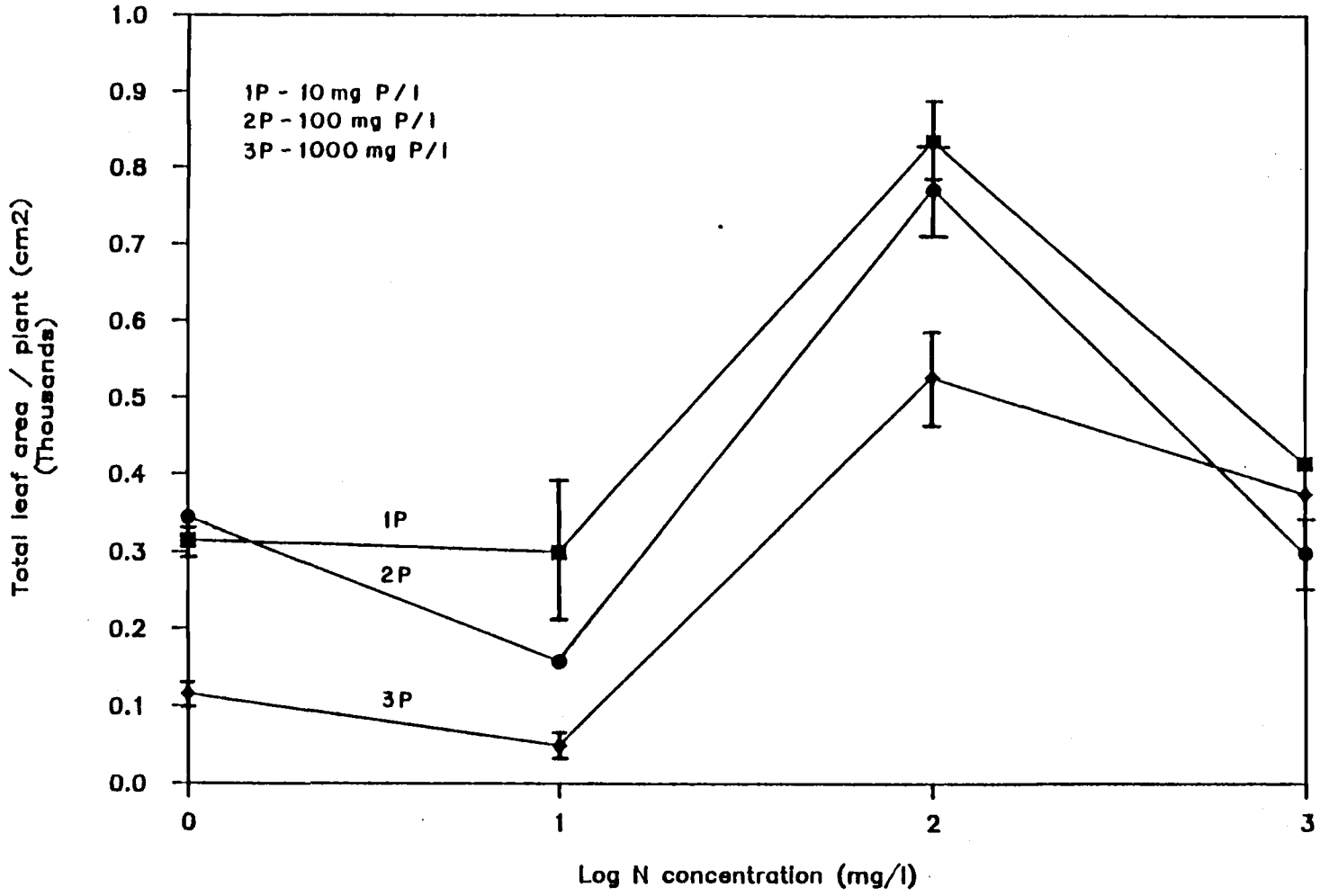

Figure 6: Total leaf area per seedling. Means and 95% confidence limits are presented. Some confidence limits are smaller than the symbols.

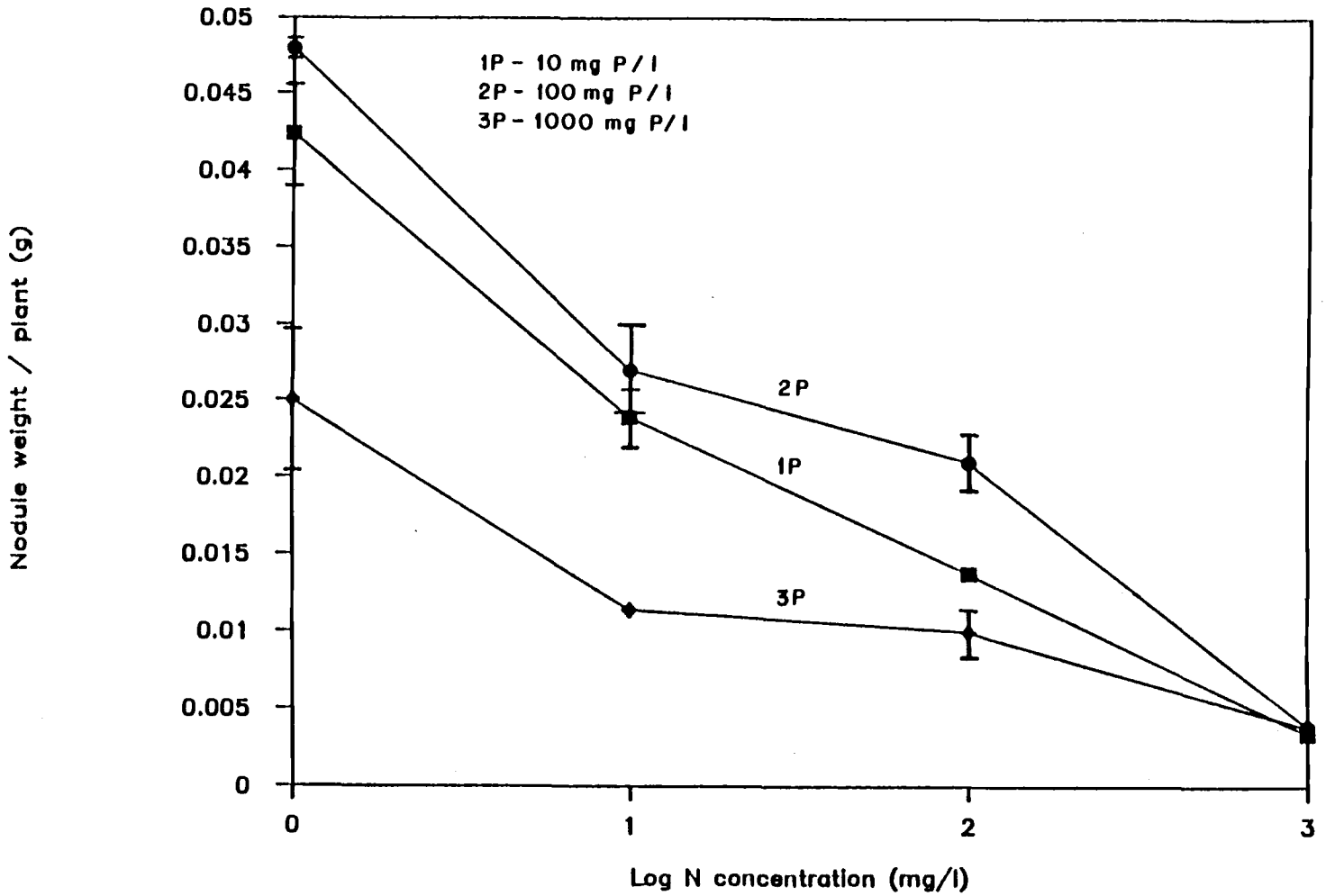

Figure 7: Nodule weight per seedling. Means and 95% confidence limits are presented. Some confidence limits are smaller than the symbols.

treatments were not significantly different ($P < 0.05$) when in combination with the 1, 10, and 1000 mg N /l treatments. Nodule biomass increased significantly ($P < 0.05$) over all other treatment combinations when N and P were combined in solution as 100 mg P/l and 100 mg N/l. Nodule biomass was depressed at solution P concentrations of 1000 mg P/l more than at 10 or 100 mg P/l. However, at N concentrations of 100 and 1000 mg N/l, the 1 and 1000 mg P/l treatments were not significantly different ($P < 0.05$). Generally, nodule biomass was enhanced when solution P concentrations reached 100 mg P/l though the positive response to 100 mg P/l was not significantly different from the response of nodule biomass to 10 mg P/l.

The relative distribution of biomass among plant parts, expressed as a percentage of total biomass, remained fairly consistent over all treatments (Table 2). The greatest proportion of dry matter production was to leaf biomass. Nodule biomass, though a very small proportion of the total biomass, notably declined with increasing N concentration. There was a decrease in the proportion of dry matter allocated to leaf biomass when solution P concentrations were 1000 mg P/l combined with 1 and 10 mg N/l. This decrease is offset by slight increase in stem and root biomass. The actual biomass distribution for each treatment is presented in Figure 8.

Table 2: Percent biomass distribution among plant parts.

Treatment (mg/l)	leaf wt. (%)	stem wt. (%)	root wt. (%)	nodule wt. (%)
1 N /10	59.2	18.4	19.7	2.7
1 N /100 P	60.3	18.6	18.3	2.8
1 N /1000 P	47.0	21.0	29.0	3.0
10 N /10 P	57.8	19.4	21.3	1.5
10 N /100 P	60.3	19.3	17.5	2.9
10 N /1000 P	34.7	28.0	34.6	2.7
100 N /10 P	57.7	25.6	16.4	0.3
100 N /100 P	55.6	29.9	14.0	0.5
100 N /1000 P	57.8	25.6	16.3	0.3
1000 N/10 P	59.6	23.5	16.7	0.2
1000 N/100 P	58.6	19.9	21.3	0.2
1000 N/1000 P	57.3	22.7	19.8	0.2

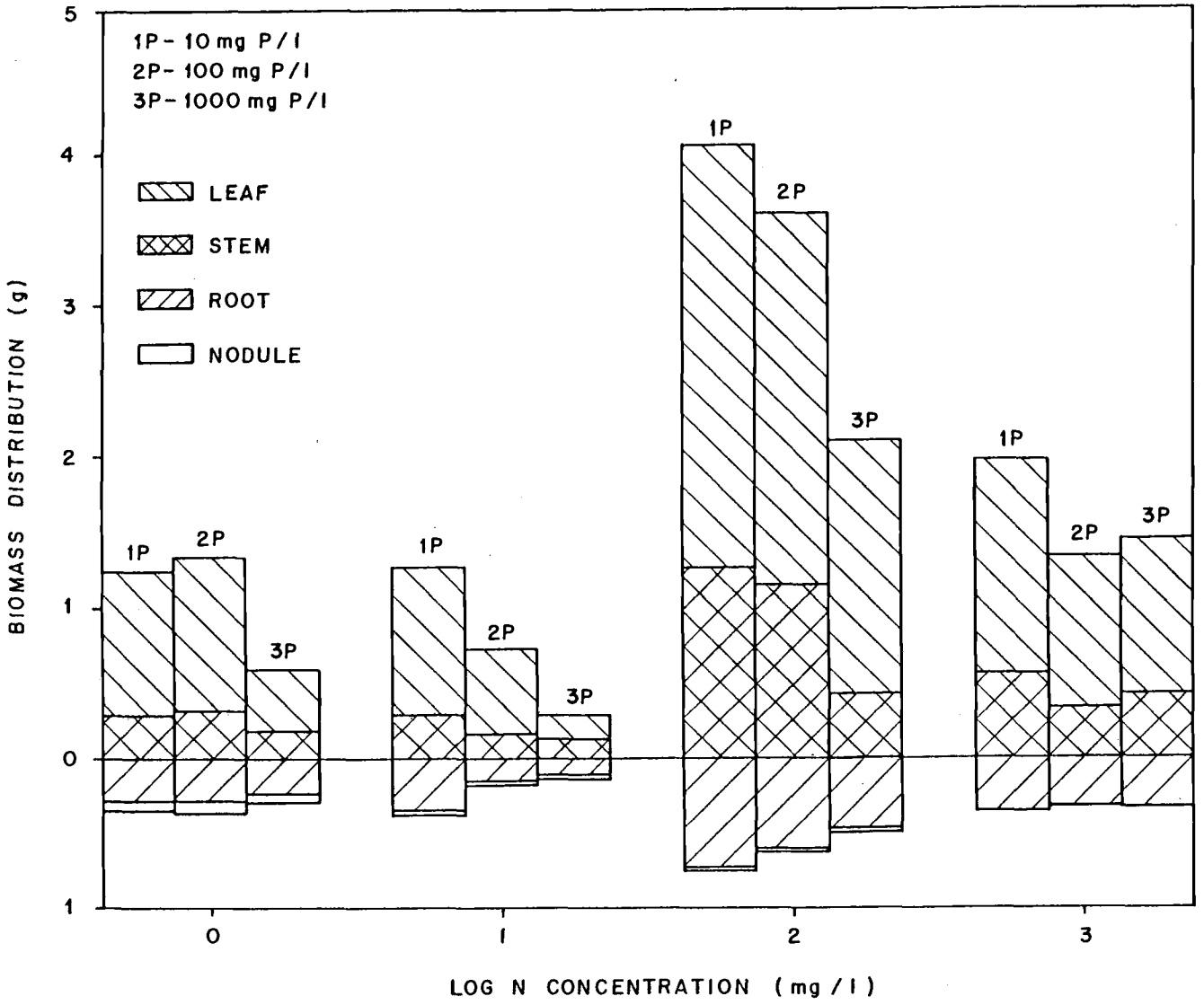

Figure 8: Biomass distribution among plant parts.

The shoot:root ratio increased slightly with increasing external N concentration but only the 100 mg N/l treatments were significantly different from the 1 and 10 mg N/l treatments (Figure 9). The 1000 mg P/l treatment was significantly different ($P < 0.05$) from the 10 and 100 mg P/l treatments when in solution with 1 and 10 mg N/l but not in combination with 100 or 1000 mg N/l. There was a significant interaction among N and P as indicated by analysis of variance (Table 1).

Correlation analyses indicated a significant positive relationship among total leaf area and leaf, stem, and root dry matter (Table 3). Only nodule biomass was not significantly correlated ($P < 0.05$) to any other plant biomass measures.

Total biomass and most other biomass measures were significantly correlated to leaf N content (Table 4). Nodule biomass was negatively correlated to both leaf N content and leaf N concentration. All biomass variables were negatively correlated to leaf P concentrations. Plant dry matter tended to decrease with increasing solution P concentrations from 10 to 100 mg P/l in solution though the treatments were not significantly different (Figure 2).

Leaf area, leaf weight, and stem weight were weakly correlated to nodule AR reduction rates (Table 4). There were no biomass variables significantly correlated with

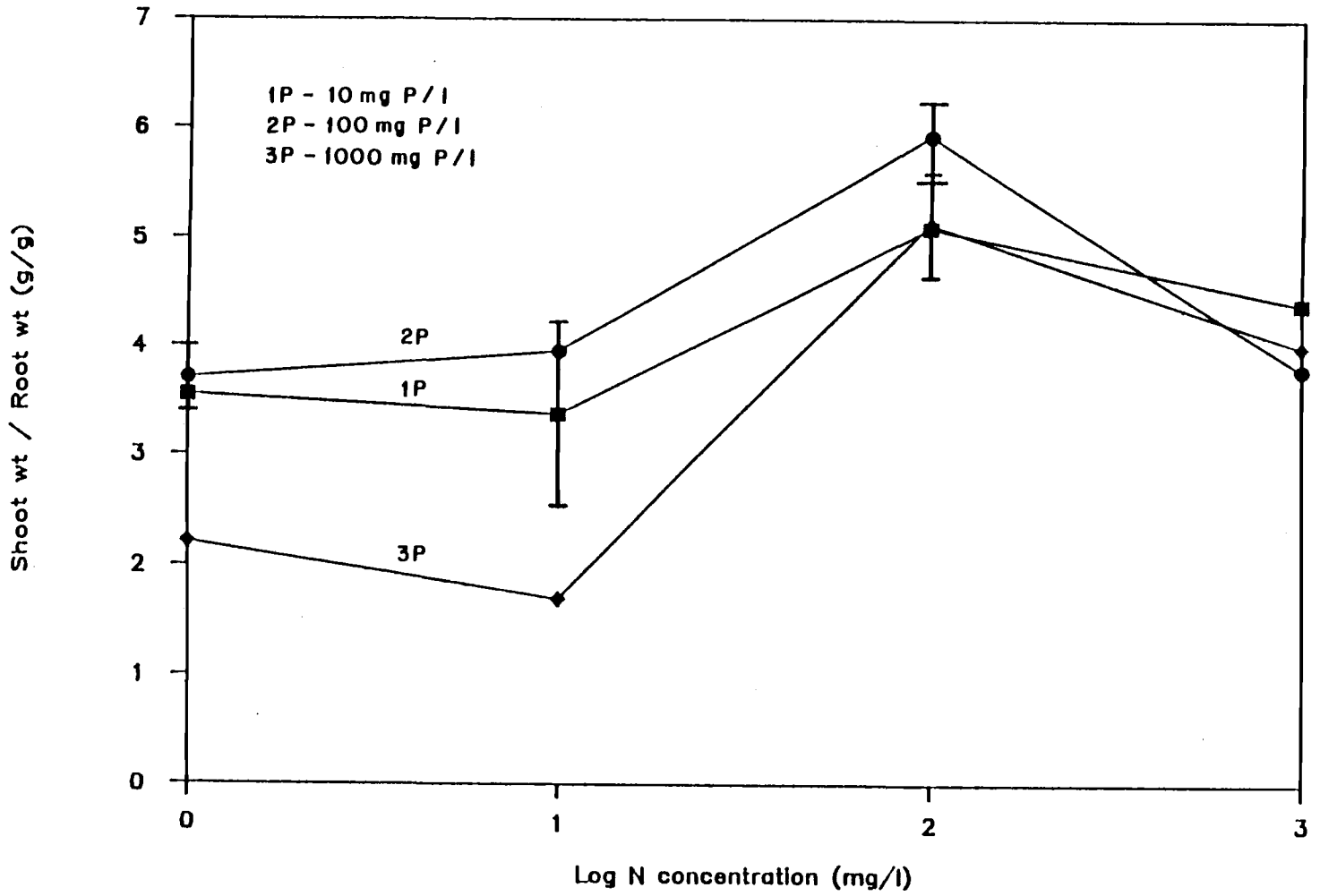

Figure 9: Shoot /Root ratio per seedling. Means and 95% confidence limits are presented. Some confidence limits are smaller than the symbols.

Table 3: Linear correlations among biomass variables for red alder after 11 weeks. Correlation coefficients above ($P < 0.05$), probabilities of greater r below. Biomass values presented in grams. Leaf area presented in cm^2.

	Leaf area	Nodule d.wt.	Root d.wt.	Stem d.wt.	Leaf d.wt.
Plant biomass d.wt.	**.96** (.0001)	**-.13** (.1950)	**.94** (.0001)	**.97** (.0001)	**.99** (.0001)
Leaf d.wt.	**.97** (.0001)	**-.12** (.2405)	**.92** (.0001)	**.92** (.0001)	
Stem d.wt.	**.89** (.0001)	**-.17** (.1175)	**.89** (.0001)		
Root d.wt.	**.89** (.0001)	**-.16** (.1175)			
Nodule d.wt.	**-.11** (.3039)				

Table 4: Linear correlations among acetylene reduction rates and biomass variables for red alder after 11 weeks. Correlation coefficients above ($P < 0.05$), probabilities of greater r below. Biomass variables presented in grams. Leaf area presented in cm^2.

	Nodule AR rate (umoles C_2H_2/ nodule/hr)	Plant AR rate (umoles C_2H_2/ plant/hr)	Leaf N content (g)	Leaf P content (g)	Leaf N (%)	Leaf P (%)
Total biomass	.25 (.0172)	- .02 (.8682)	.90 (.0001)	.38 (.0072)	.13 (.4001)	-.53 (.0001)
Leaf d.wt.	.24 (.0233)	-.01 (.9343)	.93 (.0001)	.41 (.0031)	.17 (.2291)	-.57 (.0001)
Stem d.wt.	.27 (.0103)	-.04 (.6825)	.81 (.0001)	.35 (.0135)	.03 (.8333)	-.44 (.0014)
Root d.wt.	.18 (.0884)	-.06 (.5702)	.83 (.0001)	.25 (.0780)	.13 (.3770)	-.50 (.0002)
Nodule d.wt.	.41 (.0001)	.83 (.0001)	-.29 (.0464)	-.14 (.3243)	-.43 (.0027)	-.10 (.5116)
Total leaf area	.26 (.0150)	.01 (.9483)	.92 (.0001)	.47 (.0007)	.21 (.1484)	-.55 (.0001)
% Leaf P	-.22 (.1550)	-.19 (.2211)	-.59 (.0001)	.16 (.2643)	-.37 (.0106)	
% Leaf N	-.41 (.0063)	-.29 (.0564)	.51 (.0002)	.06 (.6769)		
Leaf P content	.09 (.5550)	-.01 (.9278)	.36 (.0119)			
Leaf N content	-.02 (.8746)	-.13 (.3930)				

plant AR reduction rates, except nodule biomass, which was used to calculate plant AR reduction rates.

Acetylene reduction

Analysis of variance indicated that nodule and plant AR rates were significantly ($P < 0.001$) related to N and P treatments (Table 1). Also, there were significant interactions ($P < 0.01$) among the N and P treatments for both nodule and plant acetylene reduction rates. Acetylene reduction (AR) rates on a nodule weight basis were depressed at the highest concentrations of nitrogen and phosphorus (1000 mg N/l and 1000 mg P/l) (Figure 10, Appendix III). The 1, 10, and 100 mg N/l treatments were, generally, not significantly different from one another but were significantly different from the 1000 mg N/l treatments. The 100 mg N/l and 100 mg P/l treatment combination was significantly increased over the other N treatments. The P treatments on nodule AR rates were not significantly different from one another except for the one treatment combination of 100 mg P/l with 100 mg N/l. Generally, nodule AR rates did not respond to increasing external solution N and P concentrations.

Negative correlations were found between nodule AR rates and leaf nitrogen concentration (Table 4). There were no correlations among nodule AR rates and leaf N and

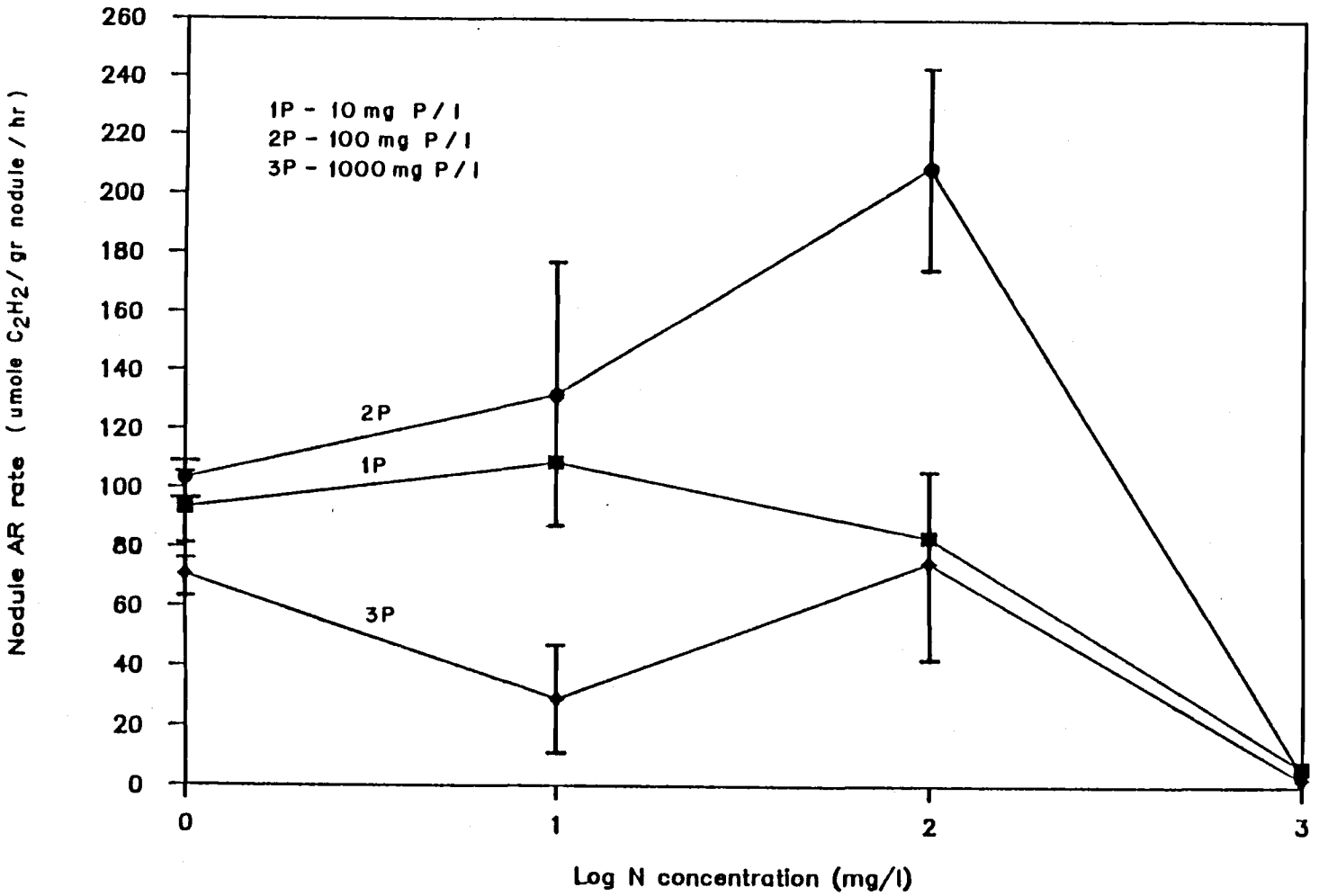

Figure 10: Nodule acetylene reduction (AR) rates. Means and 95% confidence limits are presented. Some confidence limits are smaller than the symbols.

P content or leaf P concentration.

Acetylene reduction rates on a plant basis were significantly depressed at high solution concentrations of N and P (1000 mg N/l and 1000 mg P/l) (Figure 11, Appendix III). Plant AR rates were significantly different ($P < 0.05$) for each treatment level of N in combination with 10 mg P/l. Plant AR rates, however, did not respond to increasing N concentrations from 1 to 100 mg N/l when in combination with either 100 mg P/l or 1000 mg P/l. The 100 mg P/l treatments tended to be greater than the 10 mg P/l treatments in combination with all N treatments though there was no significant difference between these two levels of P except in the combined 100 mg P/l and 100 mg N/l treatment. Plant AR rate was not significantly correlated with any other measured variable (Table 4).

Leaf nutrients

Analysis of variance indicated that leaf N content and concentration are significantly related to treatment levels of N and P (Table 1). Leaf P concentration, however, is significantly related to treatment P concentrations but not to solution N concentrations. Leaf P content was significantly related to solution N and P treatments but also had a significant N and P interaction.

Leaf nitrogen concentration remained constant over

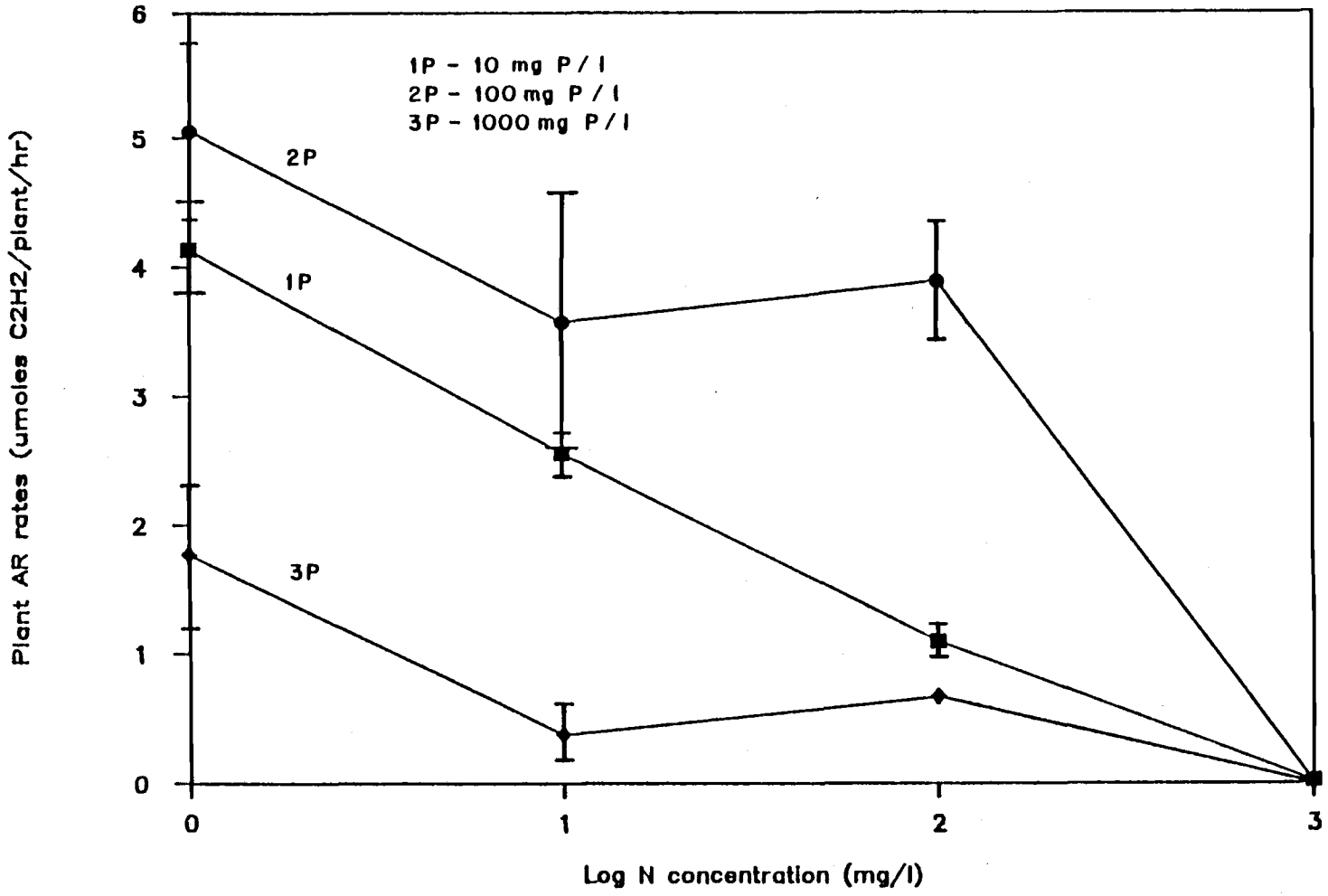

Figure 11: Plant acetylene reduction (AR) rates. Means and 95% confidence limits are presented. Some confidence limits are smaller than the symbols.

all levels of solution N except at 1000 mg N /l where leaf nitrogen concentration increased (Figure 12). Phosphorous treatments were significantly different only at 1 mg N/l.

Plant leaf nitrogen content increased when solution N concentrations increased to 100 mg N/l. The 1 and 10 mg N/l treatments were not significantly different (Figure 13). Leaf N content generally decreased with increasing external P concentrations but the only significant P treatment was the 1 mg N/l and 1000 mg P/l treatment combination.

Leaf phosphorus concentrations did not respond to increasing external N concentrations but did increase in response to increasing P treatments (Figure 14). Leaf P content increased with increasing solution P concentrations when these P treatments were in combination with 100 and 1000 mg N/l (Figure 15). At 1 and 10 mg N/l, leaf P content did not increase with increasing solution P, but remained relatively constant over all concentrations of solution P.

The change in the ratio of leaf N to leaf P at each level of N was primarily due to a change in P concentrations, as leaf N concentrations remained relatively stable over the range of external solution N concentrations from 1 to 100 mg N/l while leaf P concentrations increased with increasing solution P supply (Figure 16). This ratio indicates a shift in the balance between leaf N and P as leaf P concentrations increase. This shift may be related

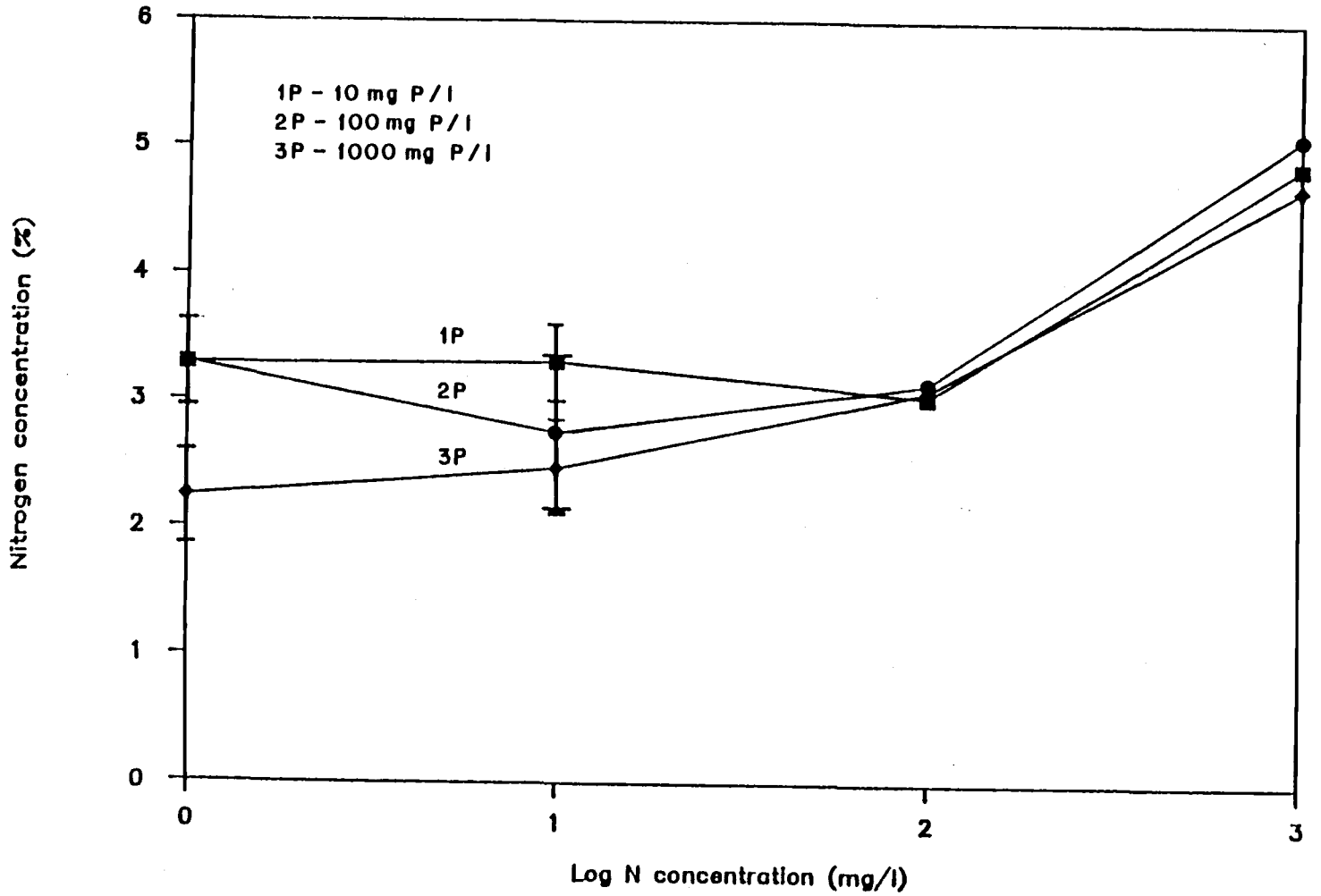

Figure 12: Leaf nitrogen concentrations. Means and 95% confidence limits are presented. Some confidence limits are smaller than the symbols.

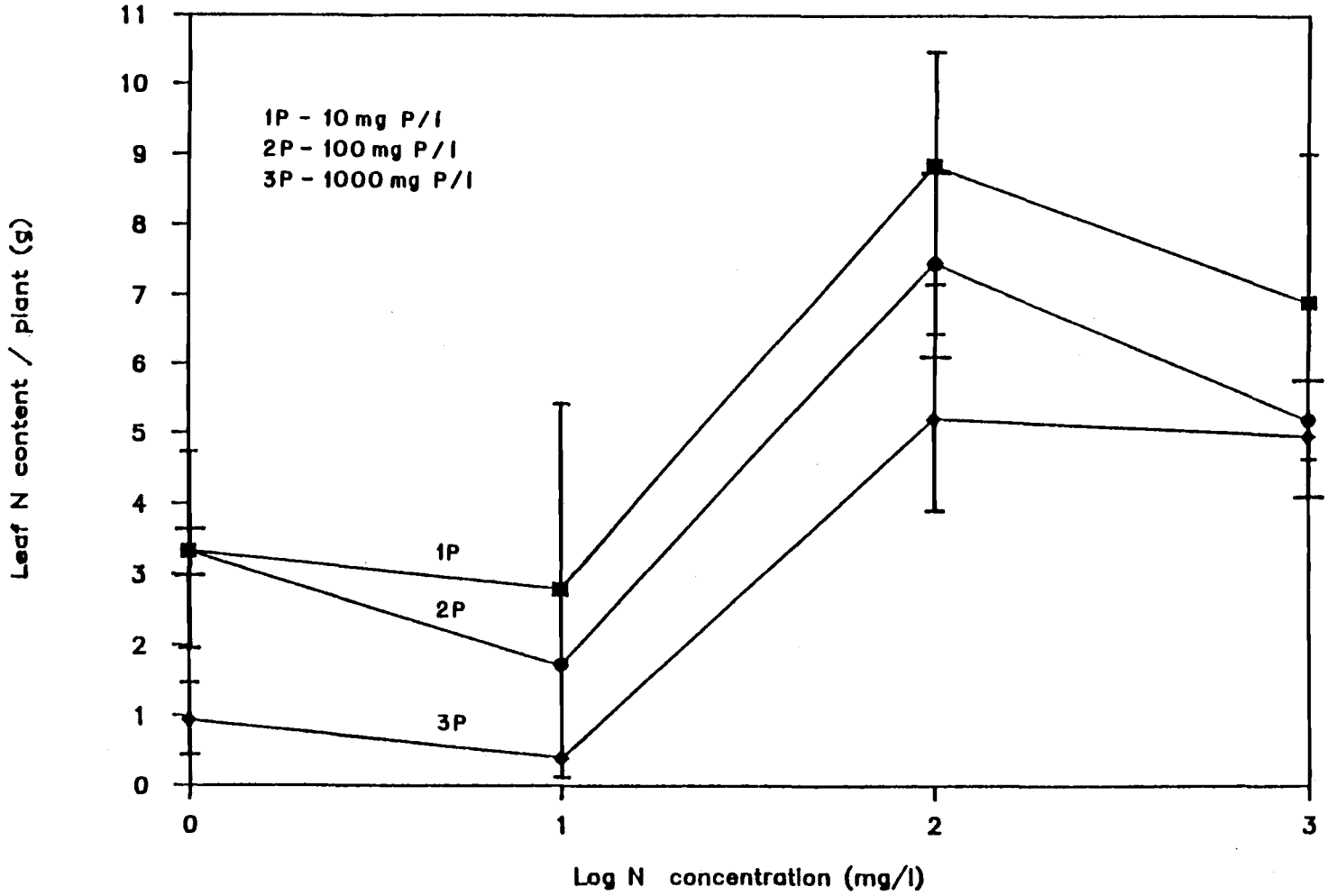

Figure 13: Leaf nitrogen content per seedling. Means and 95% confidence limits are presented. Some confidence limits are smaller than the symbols.

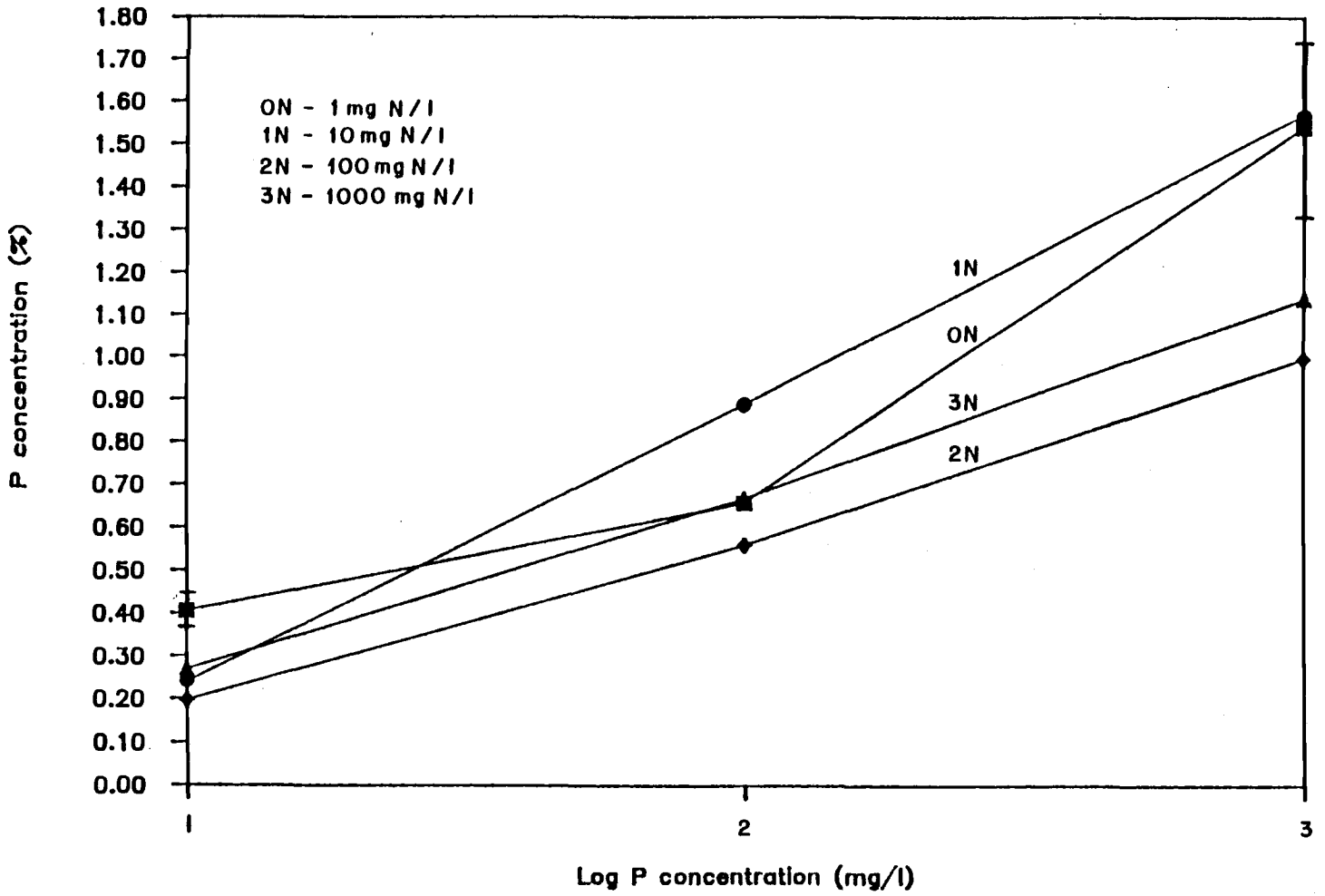

Figure 14: Leaf phosphorus concentrations. Means and 95% confidence limits are presented. Some confidence limits are smaller than the symbols.

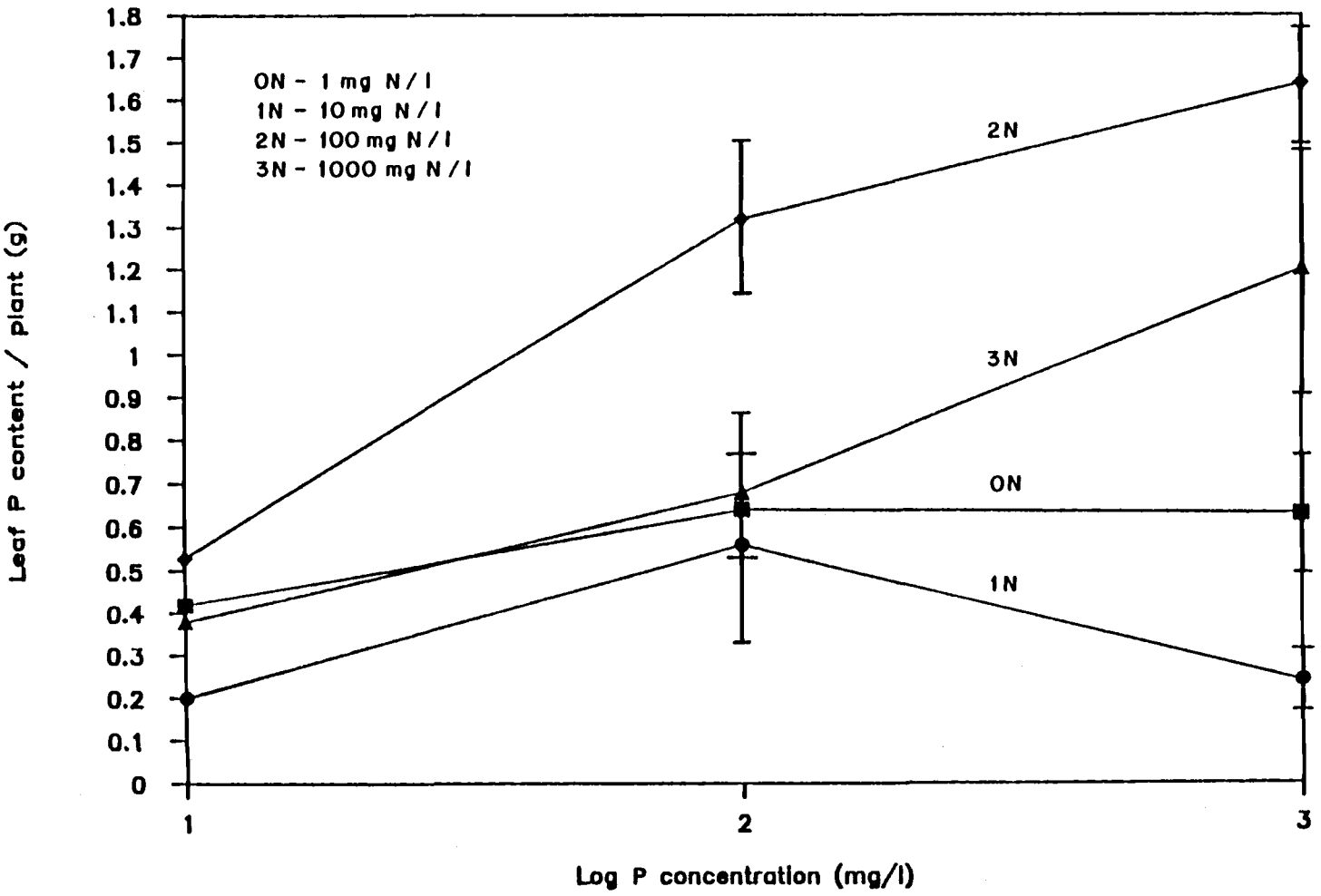

Figure 15: Leaf phosphorus content per seedling. Means and 95% confidence limits are presented. Some confidence limits are smaller than the symbols.

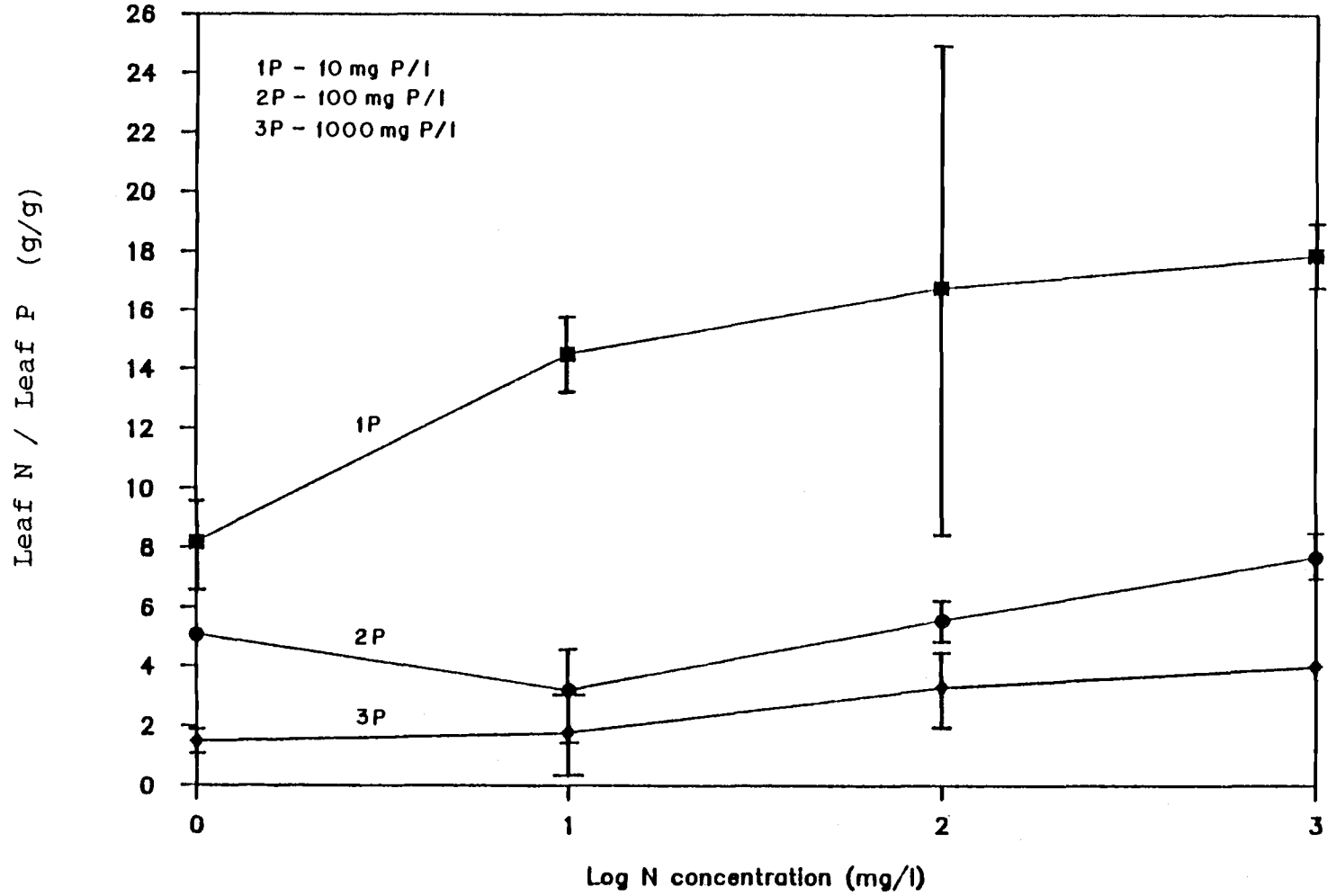

Figure 16: Leaf N / Leaf P ratio. Means and 95% confidence limits are presented. Some confidence limits are smaller than the symbols.

to decreasing plant biomass and increasing nodule biomass as solution P concentrations increase.

Sodium treatment

Solutions containing high sodium (Na) included all treatments containing 1000 mg P/l. A solution treatment was produced using high levels of a sodium compound ($NaSO_4$) which was applied to the plants in order to mimic the amount of sodium present in the 1000 mg N/l and 1000 mg P/l treatment combination. The purpose of this treatment was to determine if the response to the 1000 mg N/l and 10 mg P/l and 1000 mg N/l and 1000 mg P/l treatments were similar to the response to the 1000 mg N/l, 10 mg P/l and 1000 mg Na/l (sodium replacement) treatment. If the seedlings did not respond to the presence of sodium in the sodium replacement treatment then the response of red alder seedlings to the sodium replacement treatment would be similar to the plant response to the 1000 mg N/l and 10 mg P/l treatment. A difference in seedling response between these treatments would require an evaluation of the response of the seedlings to Na as well to P.

The sodium replacement treatment had a negative effect on almost all variables measured (Appendix II and III). Leaf and stem biomass production were significantly reduced ($P < 0.05$) by the sodium replacement treatment compared

to the reponse to the 1000 mg N/l and 10 mg P/l and the 1000 mg N/l and 1000 mg P/l treatments. Nodule biomass, leaf N and P percentage, and plant AR rates, however were slightly increased with the sodium replacement treatment than the 1000 mg N/l and 10 mg P/l and 1000 mg N/l and 1000 mg P/l treatment combinations. These results suggest that the sodium present in the 1000 mg N/l and 1000 mg P/l treatment was in a less active form in terms of plant response than as applied in the replacement sodium treatment. The mechanism by which sodium may have inhibited measured biomass variables was not determined. It appeared, however, that the roots, nodules, and nodule activity were relatively unaffected by the sodium while the leaf and stem parts of the plant were significantly affected.

DISCUSSION

Nutrient status

Total soil N and P concentrations from sites in the coastal mountain range of Oregon were determined in an earlier study to be about 4200 ppm N and 1500 ppm P at the Cascade Head Experimental Forest and 1200 ppm N and 1100 ppm P at a research site near Alsea, OR. (unpublished data). Total soil N was estimated by Cole and Newton (1986) to be between 2190 mg N/l and 3130 mg N/l in the coast range depending on the plant species present on the site. Although these ranges of soil nutrient concentrations do not exactly represent the available N and P for plant use, these values do provide a range of concentrations and so were used as a basis for setting the maximum levels of N and P to use in this study.

In this study, leaf N and P concentrations were well above the deficiency values reported by Hughes et al. (1968) and Pregent and Camire (1985). Leaf P concentrations increased with increasing solution P concentrations indicating that the seedlings were taking up the available P in solution. Values of leaf P concentrations were greater than the 0.9% P limit set as the concentration at which toxicity and adverse affects of plant growth and physiological processes begin to occur (Loneragan 1968). Clover

and lotus have been reported as having high uptake rates of P even when growth reponse to increased P became asymptotic (Hart et al. 1981b). In this study there was a marked reduction in biomass at high internal levels of P.

The contribution of fixed nitrogen to the plant nitrogen pool declined with increasing external N concentrations. Leaf N concentrations were well into the optimum range for red alder in all treatments. Stable leaf N concentrations suggest that the rate of supply of N to the plant was relatively constant. However, since leaf N content was greatest at 100 mg N/l, when plant fixation rates were low nitrogen from solution must have been taken up to supplement the decrease in nitrogen supplied by fixation. Plant biomass production paralleled changes in leaf N content.

Both N and P leaf contents were higher at solution N concentrations of 100 and 1000 mg N/l compared to other treatments. Since there was no evidence of a dilution effect of plant growth on leaf nutrient concentrations, the rate of uptake of N and P must have been the same or greater than the rate utilized by plant growth when solution concentrations of N and P were high.

A change in the ratio of leaf N to leaf P with different treatments suggests that N uptake was much more regulated than P uptake. The ratio of N to P shifted as leaf P concentration increased at a greater rate than leaf N concentration as solution concentrations of N and

P increased. This shift coincided with a small decline in total plant biomass and an increase in nodule biomass. The ratio of these nutrients within the plant may be related to the allocation of carbohydrate to the nodules.

Biomass production and allocation

The energy costs of symbiotic nitrogen fixation are sometimes found to be of a sufficiently great burden to the host plant as to reduce biomass production below that attained if the plant otherwise utilized mineral nitrogen (Schubert 1982). Stewart and Bond (1965) have suggested that alders do not attain optimum growth in the absence of mineral nitrogen because the nodules do not satisfy the plants total nitrogen requirements through nitrogen fixation. Both of these papers point out the importance of nitrogen uptake and assimilation and carbon production and allocation in the growth of N_2-fixing plants.

Biomass production was clearly enhanced at 100 mg N/l over all other treatments in this study. As leaf nitrogen concentrations remained relatively stable in all N treatments, I concluded that nitrogen, provided as fixed N and mineral N, was not limiting in any of the treatments. Therefore, in this study, biomass production was not limited by a nitrogen deficiency as was suggested in another study by Stewart and Bond (1965). However, total plant growth was

reduced at N levels below 100 mg N/l. This reduction in growth coincided with increased nodule biomass and plant AR activity, except at 1000 mg N/l were all measured variables were depressed. These results are similar to those reported by Ingestad (1980) who found that even though plant nitrogen fixation rates were high and the leaf nitrogen concentrations were optimal, plant growth was reduced probably due to allocation of available carbohydrate to the nodules.

Inadequate carbohydrate allocation to the nodules limits nodule and plant nitrogen fixation. An increase in carbohydrate production and allocation may be expected to increase nitrogen fixation activity and the production of nodules. In this study, the greater leaf area and improved N content at 100 mg N/l and 100 mg P/l represent an increase in photosynthetic surface area and a potential improvement in photosynthetic rates. Carbohydrate production may increase such that both nitrogen fixation and plant growth are enhanced. However, plant biomass and photosynthetic leaf area were also high when solution concentrations were 100 mg N/l and 10 mg P/l yet neither nodule nor plant AR activity were increased by this treatment. These results suggest that the stimulation of nodule AR activity and nodule biomass at the 100 mg N and 100 mg P/l level was controlled by the supply of P.

Studies of nitrogen fixation in legumes have reported that increased nodule biomass paralleled an increase in plant biomass as the P supply increased. In this study, nodule biomass was enhanced when P was present at 100 mg P/l in solution though plant biomass declined slightly at this P treatment concentration compared to that at 10 mg P/l. Also, the 100 mg P/l treatment appeared to have modified the negative trend of external solution N concentrations on nodule development found when solution P concentrations were 10 and 1000 mg P/l. Generally, P appeared to have greater control on nodule development and nitrogenase activity in red alder than on plant growth.

Nitrogen fixation

Two symptoms of the inhibitory effect of increasing external N on nitrogen fixation reported by other researchers are a decline in nodule biomass and a decline in nodule AR activity (Stewart 1982). Nitrogen deficiencies can occur when an external source of N is unavailable and nitrogen fixation does not provide sufficient N for plant growth. These deficiencies appear to be relieved in some plants when N is made available in low concentrations in solution. Nitrogen status is then improved and appears to stimulate an increase in photosynthesis, carbohydrate production and nitrogenase activity.

In this study, nodule biomass and plant AR activity were high at low (1 and 10 mg N/l) external N concentrations. However, depressions in nodule biomass were observed as solution nitrogen concentrations increased. The decline in nodule biomass was correlated with increasing leaf N concentration. Ingestad (1980) suggests that low internal N concentration may be less inhibitory to nodule biomass than low external N concentration. In this study, it was difficult to determine whether the decline in nodule biomass was an effect of internal or external N concentrations. In this study, nitrogenase activity or nodule AR activity was generally not affected by solution N. Internal N content, as represented by leaf N content, did not appear to affect nitrogenase activity or inhibit nitrogenase synthesis. However, nodule AR activity was increased with the 100 mg N/l and 100 mg P/l treatments suggesting that an interaction of these nutrients on nodule fixation may occur. At solution concentrations of 1000 mg N/l, nitrogenase activity was suppressed. I concluded that at these high solution nitrogen concentrations, as leaf N concentrations were also high, nitrogen was accumulating in the leaf cells. Such an accumulation can inhibit physiological processes, such as photosynthesis and carbohydrate transport processes (Beavers and Hageman 1983).

In some legumes a P deficiency impairs nodule AR activity indirectly through hindrance of leaf and shoot carbohydrate metabolism but not through action on nodule formation or function (Jakobsen 1985). An increase in nodule weight and plant AR rates with alleviation of a P deficiency has paralleled an increase in shoot growth in young pea (Bethlenfalvay et al. 1978). These conclusions are in partial agreement with this study; however, increasing solution P concentrations had a generally negative effect on biomass production and a positive effect on nodule biomass and plant and nodule AR rate. Bethlenfalvay et al. (1978) reported the young pea plants in their experiments grew exponentially and that uptake rates of nutrients had not stabilized. The plants used in this study were 4 months old and are assumed to have started in this experiment with a stable nutrient status, so that P supply may not have been as important at this stage of growth. Also, leaf P concentrations in this study were at or above optimal concentrations for growth of alder, so that P may not have been a growth limiting factor.

Summary

As solution N concentrations increased leaf N status remained stable and optimal in all N treatments.

Nitrogen fixation and plant uptake of nitrogen from solution contributed to plant nitrogen status. Plant biomass was greatest when external N concentrations were 100 mg N/l and plant nitrogen fixation was very low. Nitrogen fixation appeared to be the primary source of plant nitrogen when external nitrogen concentrations were below 10 mg N/l. I concluded that the depression in plant growth at low (1 and 10 mg N/l) external N concentrations was a result of the allocation of plant carbohydrate to nodule biomass and a reduction in carbohydrate available for plant growth processes.

Nodule biomass and plant acetylene reduction were reduced while nodule AR activity remained relatively unaffected as solution N concentrations increased. Negative correlations between leaf N concentration and content and nodule biomass suggests that the reduction in nodule biomass was partly controlled by internal N status.

Nodule biomass, nodule and plant AR activity were improved by increased P supply but there was no correlation between internal P status and these variables. However, leaf P concentrations were negatively correlated to plant biomass. I concluded that the effect of P on red alder nitrogen fixation was not through stimulation of host plant growth and carbohydrate production as suggested by Robson et al. (1981) but through a direct effect of

external P concentrations on nodule production and activity.

Though there has been extensive research on the effect of external N concentrations on nitrogen fixation in legumes very little research has been done on non-legume nitrogen-fixing plants and none on the role of P in these systems. In this study it was found that an increase in the supply of P can increase red alder nitrogen fixation even in the presence of high external N concentrations. The potential for increasing legume and non-legume N fixation with the application of P fertilizer becomes important in questions of soil amelioration and crop production.

BIBLIOGRAPHY

Akkermans, A.D., Roelofsen, L.W., Blom, J., Huss-Danell, K. and R. Harkink. 1983. Utilization of carbon and nitrogen compounds by Frankia in synthetic media and in root nodules of Alnus glutinosa, Hippophae rhamnoides, and Datisca cannabina. Can. J. Bot. 61:2793-2800

Benecke, V. 1970. Nitrogen fixation by Alnus viridis (Chaix) D.C. Plant and Soil 33:30-48

Bethlenfalvay, G.J. and D.A. Phillips. 1978. Interactions between symbiotic nitrogen fixation, combined-N application and photosynthesis in Pisum sativum. Physiol. Plant. 42:119-123

_____, Abu-Shakra, S.S. and D.A. Phillips. 1978. Interdependence of nitrogen nutrition and photosynthesis in Pisum sativum L. Plant Physiol. 62:127-130

Beavers, L. and R.H. Hageman. 1983. Uptake and reduction of nitrate: bacteria and higher plants. Encyc. of Plant Physiol. 15A:351-375

Binkley, D. 1983. Ecosystem production in Douglas-fir plantations: interactions of red alder and site fertility. For. Ecol. Manage. 5:215-227

Bond, G., Fletcher, W. and T.P. Ferguson. 1954. The development and function of the root nodules of Alnus, Myrica and Hippophae. Plant and Soil 5:309-323

Bormann, B.T. 1981. Stand density in young red alder plantations: production, photosynthate partitioning, nitrogen fixation and an optimal spacing model. Ph.D. thesis. Oregon State University. Corvallis, OR. 62 pp.

Chapin, S.F. 1980. The mineral nutrition of wild plants. Ann. Rev. Ecol. Syst. 11:233-260

Cole, E.C. and M. Newton. 1986. Nutrient, moisture, and light relations in 5-yr-old Douglas-fir plantations under variable competition. Can. J. For. Res. 16: 727-732

Date, R.A. 1973. Nitrogen, a major limitation in the productivity of natural communities, crops and pastures in the Pacific area. Soil Biol. Biochem 5:5-18

Dawson, J.O. and J.C. Gordon. 1979. Nitrogen fixation in relation to photosynthesis in Alnus glutinosa. Bot. Gaz. 140:S70-S75

Dietz, K.-J. and C. Foyer. 1986. The relationship between phosphate status and photosynthesis in leaves. Planta 167: 376-381

Finke, R.L. , Harper, J.E.and R.H. Hageman. 1982. Efficiency of nitrogen assimilation of N-fixing and nitrate-grown soybean plants (Glycine max (L.) Merr.). Plant Physiol. 71:1178-1184

Gates, C.T. and J.R. Wilson. 1974. The interaction of nitrogen and phosphorous on the growth, nutrient status, and nodulation of Stylosanthes humilis H.B.K. (Townsville Stylo). Plant and Soil 41:325-333

Gibson, A.H. and J.D. Pagan. 1977. Nitrate effects on the nodulation of legumes inoculated with nitrate reductase-deficient mutants of Rhizobium. Planta 134:17-22

Gifford, R.M. and L.T. Evans. 1981. Photosynthesis, carbon partitioning and yield. Ann. Rev. Plant Physiol. 32: 485-509

Gordon, J.C. 1978. Biological components of alder yield improvement. In D.G. Briggs, D.S. Debell and W.A. Atkinson, (eds.). Utilization and management of alder. USDA For. Ser. Gen. Tech. Rep. PNW-70, Portland, OR. p. 321-325

_____ and C.T. Wheeler. 1978. Whole plant studies on photosynthesis and acetylene reduction in Alnus glutinosa. New Phytol. 80:179-186

Gordon, A.J., Ryle, G.J.A., Mitchell, D.F. and C.E. Powell. 1985. The flux of ^{14}C-labelled photosynthate through soyabean root nodules during N_2 fixation. J. Exp. Bot. 36:756-769

Gutschick, V.P. 1978. Energy and nitrogen fixation. Bioscience 28:571-575

Hansen, E.A. and J.O. Dawson. 1982. Effect of Alnus glutinosa on hydrid Populus height growth in a short-rotation intensely cultured plantation. For. Sci. 28:49-59

Hardy, R.W.F. and U.D. Havelka. 1976. The role of photo-assimilates in nitrogen fixation in soybean. In P. Nutman (ed.). Symbiotic nitrogen fixation in plants. Int. Biol. Prog. Series Vol. VII. Cambridge University Press:London. pp. 421-439

Hardy, R.W.F., Burns, R.C. and R.D. Holsten. 1973. Applications of the acetylene-ethylene assay for measurement of nitrogen fixation. Soil Biol. Biochem. 5:47-81

Hart, A.L., Jessop, D.J. and J. Galpin. 1981a. The response to phosphorous of white clover and lotus inoculated with rhizobia or given KNO_3. N.Z. Journal of Agricultural Research 24:27-32

Hart, A.L., Halligan G. and R.M. Haselmore. 1981b. Analysis of the response of pasture legumes to phosphorous in a controlled environment. N.Z. Journal of Agricultural Research 24:27-32

Hayes, L., Torrey, J.G. and P. Young. 1985. Asparagine and glutamine as nitrogen sources controlling the development and nitrogenase activity of Frankia sp., strain HFPCpI1. In H.J. Evans, P.J. Bottomley and W.E. Newton (eds.). Nitrogen fixation research in progress. Marinus Nijhoff Publishers:The Hague. p.365.

Heldt, H.W., Chon, C.J., Maronde, D., Herold, A. Stankovic, Z.S., Walker, D.A. Kraminer, A., Kirk, M.R. and U. Heber. 1977. Role of orthophosphate and other factors in the regulation of starch formation in leaves and isolated chloroplasts. Plant Physiol. 59:1146-1155

Helgerson, O.T. 1981. Nitrogen fixation by scotch broom (Cytisus scoparius L.) and red alder (Alnus rubra Bong.) planted under precommercially thinned Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco). Ph.D. thesis. Oregon State University, Corvallis, OR. 81 p.

_____, Gordon, J.C. and D.A. Perry. 1984. N_2 fixation by red alder (Alnus rubra) and scotch broom (Cytisus scoparius) planted under precommercially thinned Douglas-fir (Pseudotsuga menziesii). Plant and Soil 78:221-233

Hielman, P. and R.F. Stettler. 1983. Phytomass production in young mixed plantation of Alnus rubra (Bong.) and cottonwood in western Washington. Can. J. Microbiol. 29:1007-1013

Hoagland, D.R. and D.I. Arnon. 1938. The water culture method for growth of plants without soil. Calif. Agr. Exp. Sta. Circ. 347.

Huber, S.C., Doehlert, D.C., Kerr, P.S. and W. Kalt-Torres. 1985. Regulation of photosynthetic sucrose formation in leaves. In P.W. Ludden and J.E. Burris (eds.). Nitrogen fixation and CO_2 metabolism. Elsevier: New York. pp. 399-407

Hughes, D.R., Gessel, S.P. and R.B. Walker. 1968. Red alder deficiency symptoms and fertilizer trials. In J.M. Trappe, J.F. Franklin, R.F. Tarrant and G.M. Hansen (eds.). Biology of alder. PNW For. and Range Exp. Station, Portland, OR. pp. 225-237

Huss-Danell, K., Roelofsen, W., Akkermans, A.D.L. and P. Jeuer. 1982. Carbon metabolism of Frankia spp. in root nodules of Alnus glutinosa and Hippophaë rhamnoides. Physiol. Plant. 54:461-466

_____, and A. Sellstedt. 1983. Nitrogenase activity in response to restricted shoot growth in Alnus incana. Can. J. Bot. 61:2949-2955

_____ and A. Sellstedt. 1985. Nitrogenase activity in response to darkening and defoliation of Alnus incana. J. Exp. Bot. 36:1352-1358

Ingestad, T. 1971. A definition of optimum nutrient requirements in birch seedling. II. Physiol. Plant. 24:118-125

_____. 1980. Growth, nutrition, and nitrogen fixation in grey alder at varied rate of nitrogen addition. Physiol. Plant. 50:353-364.

Imsande, J. 1986. Inhibition of nodule development in soybean by nitrate or reduced nitrogen. J. Exp. Bot. 37:348-355

Israel, D.W. 1985. Effect of phosphorous nutrition on symbiotic dinitrogen fixation in soybean plants. In H.J. Evans, P.J. Bottomley and W.E. Newton (eds.). Nitrogen fixation research in progress. Martinus Nijhoff Publishers:The Hague. p. 317

Jakobsen, I. 1985. The role of phosphorous in nitrogen fixation by young pea plants (Pisum sativum). Physiol. Plant. 64:190-196

Kouchi, H., Yoneyama, T. and S. Akao. 1986. Compartmental analysis of the partitioning of photo-assimilated carbon in nodulated soybean plants during the light period. J. Exp. Bot. 37:994-1005

Lambers, H., Layzell, D.B. and J.S. Pate. 1980. Efficiency and regulation of root respiration in a legume: Effects of the N-source. Physiol. Plant. 50:319-325

Leaf, G., Gardner, I.C. and G. Bond. 1958. Observations on the composition and metabolism of the nitrogen-fixing root nodules of Alnus. J. Exp. Bot. 27:320-331

Loneragan, J.F. 1968. Nutrient reguirements of plants. Nature 220:1307-1308

Lopez, M., Young, P. and J.G. Torrey. 1986. A comparison of carbon source utilization for growth and nitrogenase activity in two Frankia isolates. Can. J. Microbiol. 32:353-358

MacConnell, J.T. and G. Bond. 1957. A comparison of the effect of combined nitrogen on nodulation in non-legumes and legumes. Plant and Soil 8:378-388

Mahon, J.D. 1983. Energy relationships. In W.J. Broughton (ed.). Nitrogen fixation, vol. 3. Clarendon Press: Oxford. pp. 299-335

McLachlan, K.D. and B.W. Norman. 1961. Phosphorus and symbiotic nitrogen fixation in subterranean clover. J. Aust. Inst. Agric. Sci. 27:244-245

Marcus-Wyner, L. and D.W. Rains. 1983. Patterns of ammonium absorption and acetylene reduction during soybean developmental growth. Physiol. Plant. 59:79-82

Meesters, T.M., van Genesen, S.T. and A.D.C. Akkermans. 1985. Growth, acetylene reduction activity and localization of nitrogenase in relation to vesicle formation in Frankia strains Cc1.17 and Cp1.2. Arch. Microbiol. 143:137-142

Miller, R.E. and M.D. Murray. 1978. The effects of red alder on growth of Douglas-fir. In D.G. Briggs, D.S. Debell and W.A. Atkinson (eds). Utilization and management of alder. PNW For. and Range Exp. Station, Portland, OR. pp. 283-306

Minchin, F.R. and J.S. Pate. 1973. The carbon balance of a legume and the functional economy of its root nodules. J. Exp. Bot. 24:259-271

Minchin, F.R., Summerfield, R.J. and M.C. Neues. 1980. Carbon metabolism, nitrogen assimilation, and seed yield of cowpea (Vigna unguiculata L. Walp.) grown in an adverse temperature regime. J. Exp. Bot. 31: 1327-1345

Monaco, P.A., Ching, T.M. and K.K. Ching. 1981. Variation of Alnus rubra for nitrogen fixation capacity and biomass production. Silvae Genetica 30:46-50

Moorby, J. and R.T. Besford. 1980. Mineral nutrition and growth. In A. Läuchli and R.L. Bieleski (eds.). Inorganic plant nutrition. Springer Verlag:New York. pp. 481-527.

Noel, K.D., Carneol, M. and W.J. Brill. 1982. Nodule protein synthesis and nitrogenase activity of soybeans exposed to fixed nitrogen. Plant Physiol. 70:1236-1241

Nordmeyer, A.H. 1969. Effects of nitrogen on fixation activity. New Zealand Forest Serv. Rep. 12:57-60.

Pate, J.S., Layzell, D.B. and C.A. Atkins. 1979. Economy of carbon and nitrogen in a nodulated and non-nodulated (NO_3-grown) legume. Plant Physiol. 64:1083-1088

Paul, E.A. and R.M. Kucy. 1981. Carbon flow in plant microbial associations. Science 213:473-474

Peterson, R.G. 1985. Design and analysis of experiments. Marcel Dekker:New York. 429 p.

Pregént, G. and C. Camiré. 1985. Mineral nutrition, dinitrogen fixation, and growth of Alnus crispa and Alnus glutinosa. Can. J. For. Res. 15:855-861

Quebedeaux, B. 1979. Symbiotic N_2 fixation and its relationship to photosynthetic carbon fixation in higher plants. In M.Gibbs and E. Latzko (eds.). Photosynthesis II: Photosynthetic carbon metabolism and related processes. Encyc. of Plant Physiol. 6:472-480

Quispel, A. 1954. Symbiotic nitrogen fixation in non-leguminous plants. II. The influence of inoculation density and external factors on the nodulation of Alnus glutinosa and its importance to our understanding of the mechanism of infection. Acta. Botan. Neerl. 3:512-531

_____. 1983. Dinitrogen-fixing symbioses with legumes, non-legume angiosperms and associative symbioses. In A. Läuchli and R.L. Bieleski (eds.). Inorganic plant nutrition. Encyc. of Plant Physiol. 15A:286-329

Robinson, M. and C. Baysdorfer. 1985. Interrelationships between photosynthetic carbon and nitrogen metabolism in mature soybean leaves and isolated leaf mesophyll cells. In R.L. Heath and J. Preiss (eds.). Regulation of carbon partitioning in photosynthetic tissue. University of California Press:Riverside. pp. 333-354

Robson, A.D., O'Hara, G.W. and L.K. Abbott. 1981. Involvement of phosphorous in nitrogen fixation by subterranean clover (Trifolium subterraneum L.). Aust. J. Plant Physiol. 8:427-436

Russell, S.A., Evans, H.E. and P. Mayeux. 1968. The effect of cobalt and certain other trace metals on the growth and vitamin B_{12} content of Alnus rubra. In J.M. Trappe, J.F. Franklin, R.F. Tarrant and G.M. Hansen (eds.). Biology of alder. PNW For. and Range Exp. Station, Portland, OR. pp. 259-272

Ryle, G.L.A., R.A. Arnott, C.E. Powell and A.J. Gordon. 1984. N fixation and the respiratory costs of nodules, nitrogenase activity, and nodule growth and maintenance in Fiskeby soybean. J. Exp. Bot. 35:1156-1165.

Schubert, K.R. 1986. Products of biological nitrogen fixation in higher plants: synthesis, transport and metabolism. Ann. Rev. Plant Physiol. 37:539-574

Seiler. J.R. and L.H. McCormick. 1981. The effect of nitrogen and phosphorous on the growth and mineral nutrition of alder. Can. J. For. Res. 12:576-580

Sellstedt, A. 1986. Nitrogen and carbon utilization in Alnus incana fixing N_2 or supplied with NO_3^- at the same rate. J. Exp. Bot. 37:786-797

Sellstedt, A., Huss-Danell, K. and A. Ahlqvist. 1986. Nitrogen fixation and biomass production in symbioses between Alnus incana and Frankia strain with different hydrogen metabolism. Physiol. Plant. 66: 99-107

_____. and K. Huss-Danell. 1985. Biomass production and nitrogen utilization by Alnus incana when grown on N_2 or NH_4^+. In H.J. Evans, P.J. Bottomley and W.E. Newton (eds.). Nitrogen fixation research in progress. Martinus Nijhoff Publishers:The Hague. p. 369.

_____. and K. Huss-Danell. 1986. Biomass production and nitrogen utilization by Alnus incana when grown on N_2 or NH_4^+ made available at the same rate. Planta 167:387-394.

Silsbury, J.H. 1977. Energy requirement for symbiotic nitrogen fixation. Nature 287:11-12

_____, Catchpoole, D.W. and W. Wallace. 1986. Effects of nitrate and ammonium on nitrogenase (C_2H_2 reduction) activity of swards of subterranean clover, Trifolium subterraneum L. Aust. J. Plant Physiol. 13: 257-273

Smith, M., Hays, R.R., Clawitter, J.L. and D.W. Emerich. 1985. Effect of nitrate on the enzyme levels of Rhizobium japonicum bacteriods. In H.J. Evans, P.J. Bottomley and W.E. Newton (eds.). Nitrogen fixation research in progress. Martinus Nijhoff Publishers:The Hague. p. 332.

Smith, N.J. 1983. Effects of density stress and soil productivity on size, mortality and nitrogen fixation in artificial populations of seedling red alder (Alnus rubra Bong.). Ph.D. thesis, Oregon State University. Corvallis, OR. 144 pp.

Smith, S.E. and M.J. Daft. 1977. Interactions between growth, phosphate content and nitrogen fixation in mycorrhizal and non-mycorrhizal Medicago sativa. Aust. J. Plant Physiol. 4:403-413

Sprent, J.I. and F.R. Minchin. 1983. Environmental effects of the physiology of nodulation and nitrogen fixation. In D.G. Jones and D.R. Davies (eds.). Temperate legumes. Pitman:London. pp. 269-318

SAS Institute, Inc. 1985. SAS/STAT guide for personal computers, Version 6 edition. SAS Institute Inc.: Cary, N.C. 378 pp.

Stewart, W.D.P. 1982. Nitrogen fixation: Present and future. Israel J. Bot. 31:5-44

_____. and G. Bond. 1961. The effect of ammonium nitrogen on fixation of elemental nitrogen in Alnus and Myrica. Plant and Soil 14:347-359

Streeter, J.G. 1981. Effect of nitrate in the rooting medium on carbohyrate composition of soybean nodules. Plant Physiol. 68:840-844

_____, J.G. 1985. Nitrate inhibition of legume nodule growth and activity. II. Short term studies with high nitrate supply. Plant Physiol. 77:325-328

Tarrant, R.F. 1968. Some effects of alder on the forest environment. In J.M. Trappe, J.F. Franklin, R.F. Tarrant and G.M. Hansen (eds.). Biology of alder. PNW For. and Range Exp. Station. Portland, OR. p. 193

Tjepkema, J.D. 1985. Utilization of photosynthate for nitrogen fixation in seedlings of Myrica gale and Alnus rubra. In P.W. Ludden and J.E. Burris (eds.). Nitrogen and CO_2 metabolism. pp. 183-192

_____. and L.J. Winship. 1980. Energy requirements for nitrogen fixation in actinorhizal and legume root nodules. Science 209:279-281

_____. and C.R. Schwinter. 1986. Physiology of actinorhizal nodules. Ann. Rev. Plant Physiol. 37: 209-232

Tripp, L.N., Bezdicek, D.F. and P.E. Hielman. 1979. Seasonal and diurnal pattern and rates of nitrogen fixation of young red alder. For. Sci. 25:371-380

Troelstra, S.R. 1985. Effects of N source on protein excretion, ionic balance and growth of Alnus glutinosa (L.) Gaertner: comparison of N_2 fixation with single and mixed sources of NO_3 and NH_4. Plant and Soil 84:361-385

Wheeler, C.T. 1971. The causation of the diurnal changes in nitrogen fixation in the nodules of Alnus glutinosa. New Phytol. 70:487-495

Wheeler, C.T. and A.C. Lawrie. 1976. Nitrogen fixation in root nodules of alder and pea in relation to supply of photosynthetic assimilates. In P.S. Nutman (ed.). Symbiotic nitrogen fixation in plants. IBP 7. Cambridge University Press:Cambridge. pp.497-513

Wheeler, C.T. and M.E. McLaughlin. 1979. Environmental modulation of nitrogen fixation in actinomycete nodulated plants. In J.C. Gordon, C.T. Wheeler and D.A. Perry (eds.). Symbiotic nitrogen fixation in the management of temperate forests. Forest Research Laboratory, Oregon State University, Corvallis, OR. pp.124-142

Wheeler, C.T., Hooker, J.E., Crowe, A. and A.M.M. Berrie. 1986. The improvement and utilization in forestry of nitrogen fixation by actinorhizal plants with special reference to Alnus in Scotland. Plant and Soil 90:393-406

Winter, H.C. and R.H. Burris. 1968. Stoichiometry of adenosine triphosphate requirement for N_2 fixation and H_2 evolution by a partially purified preparation of Clostridium pasteurianum. J. Biol. Chem. 243: 940-944

Zavitkovski, J. and M. Newton. 1968. Effect of organic matter and combined nitrogen on nodulation and nitrogen fixation in red alder. In J.M. Trappe, J.F. Franklin, R.F. Tarrant and G.M. Hansen (eds.). Biology of alder. PNW For. and Range Exp. Station. Portland, OR. pp. 209-223

APPENDICES

APPENDIX I: Composition of nutrient solutions (mmoles/l)

Compound	Treatments (mg/l) 1 N P10 / P100 / P1000			10 N P10 / P100 / P1000		
NH_4NO_3	0.025	0.025	0.025	0.25	0.25	0.25
NO_3	0.021	0.0214	0.0214	0.214	-	-
H_2PO_4	-	-	-	-	1.22	28.0
$Ca(H_2PO_4)_2$	0.16	1.0	2.0	0.16	1.0	2.0
$CaSO_4$	1.84	1.0	-	1.84	1.0	-
$Ca(NO_3)_2$	-	-	-	-	-	-
KNO_3	-	-	-	-	0.21	0.214
KCl	0.5	0.5	0.5	0.5	0.5	0.5
$MgSO_4$	1.0	1.0	1.0	1.00	1.0	1.0
K_2SO_4	1.25	1.25	1.25	1.25	1.14	1.14
$NH_4H_2PO_4$	-	-	-	-	-	-

	100 N 10 P/ 100 P/ 1000 P			1000 N 10 P/ 100 P/ 1000 P		
NH_4NO_3	2.5	2.5	-	25.0	25.0	25.0
$NaNO_3$	2.14	-	-	14.9	14.9	14.9
NaH_2PO_4	-	1.22	25.7	0.322	3.22	32.2
$Ca(H_2PO_4)_2$	0.16	1.0	2.0	-	-	-
$CaSO_4$	1.84	1.0	-	-	-	-
$Ca(NO_3)_2$	-	-	-	2.0	2.0	2.0
KNO_3	-	2.14	2.14	2.5	2.5	2.5
KCl	0.5	0.9	0.9	0.5	0.5	0.5
$MgSO_4$	1.0	1.0	1.0	1.0	1.0	1.0
K_2SO_4	1.25	-	-	-	-	-
$NH_4H_2PO_4$	-	-	2.5	-	-	-

Appendix II: Means and confidence limits of biomass variables. Means with 95 % confidence limits in parentheses (n=8). Different letters indicate significantly different ($P < .05$) means within a column. Comparison of P treatments within constant N treatments designated with the letters a,b and c. Comparison of N treatments within constant P treatments designated with the letters d,e,f, and g. Biomass variables are dry weight measures.

Treatment (mg/l)	Biomass (g)	Leaf (g)	Stem (g)	Root (g)	Nodule (g)	Shoot/Root (g/g)	Leaf area cm^2
1 N / 10 P	1.60 ae (.09)	0.95 ae (.04)	0.30 ae (.02)	0.32 ae (.03)	0.0432 ad (.0033)	3.55 aef (.11)	315.41 ae (25.77)
1 N / 100 P	1.69 ae (.25)	1.02 ae (.13)	0.32 ae (.10)	0.31 ad (.03)	0.0481 ad (.0005)	3.71 ae (.34)	345.22 af (22.91)
1 N / 1000 P	0.84 bg (.13)	0.39 bf (.06)	0.18 af (.02)	0.24 af (.05)	0.0247 bd (.0047)	2.21 bf (.24)	115.80 bf (13.42)
10 N / 10 P	1.63 ae (.51)	0.95 ae (.36)	0.32 ae (.10)	0.35 ae (.05)	0.0239 ae (.0024)	3.37 af (.81)	300.09 ae (101.25)
10 N / 100 P	0.94 abde (.09)	0.57 af (.06)	0.18 abe (.02)	0.16 bf (.02)	0.0270 ae (.0031)	3.96 abe (.03)	158.01 ae (.60)
10 N / 1000 P	0.43 bf (.04)	0.15 af (.00)	0.12 bf (.02)	0.15 bg (.02)	0.0114 be (.0019)	1.70 bf (.13)	49.41 bf (12.88)
100 N / 10 P	4.83 ad (1.13)	2.76 ad (.44)	1.25 ac (.46)	0.79 ae (.22)	0.0138 bf (.0003)	5.06 ad (.27)	834.12 ad (54.08)
100 N / 100 P	4.43 ad (.25)	2.47 abd (.17)	1.33 ac (.07)	0.62 ae (.07)	0.0210 ae (.0017)	5.94 ad (.35)	772.78 ad (65.12)
100 N / 1000 P	2.94 ad (.11)	1.70 ad (.38)	0.75 ac (.08)	0.48 be (.01)	0.0102 ae (.0018)	5.14 ad (.45)	526.95 bd (56.26)
1000 N / 10 P	2.36 ae (.06)	1.41 ae (.34)	0.55 acd (.01)	0.40 af (.02)	0.0034 ag (.0005)	4.94 ade (.04)	414.79 ae (15.53)
1000 N / 100 P	1.75 be (.07)	1.03 be (.00)	0.35 bd (.04)	0.37 af (.03)	0.0039 af (.0005)	3.79 be (.11)	299.38 ae (14.40)
1000 N / 1000 P	1.85 bf (.04)	1.06 be (.01)	0.42 bd (.02)	0.37 af (.03)	0.0038 ae (.0003)	4.00 be (.26)	374.56 ae (51.06)
1000 N / 10 P / 1000 Na	0.98 c (.27)	0.47 c (.20)	0.22 c (.05)	0.28 a (.23)	0.0052 a (.0008)	2.43 c (.70)	123.27 b (51.08)

Appendix III: Means and 95% confidence limits for red alder nodule and plant acetylene reduction rates (n=8) and leaf N and P content (n=4). Different letters within a column indicate significantly different ($P < 0.05$) means. Comparison of P treatments within constant N treatments are designated with the letters a, b, and c. Comparison of N treatments within constant P treatments are designated with the letters d, e, f,and g.

Treatment (mg/l)	Nodule AR rates umoles C_2H_2/ gr nodule/hr	Plant AR rates umoles C_2H_2/ plant/hr	Leaf N (%)	Leaf P (%)	Leaf N content (g)	Leaf P content (g)	Leaf N/ Leaf P
1 N / 10 P	93.55 ade (12.20)	4.15 ad (.35)	3.30 ae (.37)	0.41 bd (.05)	3.34 ae (.34)	0.42 ade (.07)	8.2 (1.6)
1 N / 100 P	103.15 aef (10.60)	5.06 ad (.71)	3.33 ae (.10)	0.66 be (.02)	3.30 aef (1.43)	0.65 ae (.28)	5.1 (.02)
1 N / 1000 P	70.84 ad (6.95)	1.77 bd (.52)	2.25 be (.38)	1.54 ad (.22)	0.95 be (.46)	0.63 af (.12)	1.5 (.4)
10 N / 10 P	108.85 ad (2.63)	2.57 ae (.16)	3.31 ae (.35)	0.24 ae (.03)	2.80 ae (2.75)	0.20 ae (.20)	14.5 (1.4)
10 N / 100 P	132.07 ade (47.99)	3.58 ad (.98)	2.75 ae (.66)	0.89 ad (.24)	1.72 af (.13)	0.56 ae (.30)	3.2 (1.5)
10 N / 1000 P	29.07 ad (18.65)	0.38 be (.21)	2.47 ae (.79)	1.57 ad (.86)	0.39 ae (.04)	0.24 ag (.12)	1.8 (1.3)
100 N / 10 P	83.83 be (2.43)	1.10 bf (.12)	3.04 ae (.43)	0.20 ce (.11)	8.51 ad (2.0)	0.53 bd (.11)	16.8 (8.3)
100 N / 100 P	209.50 ad (35.29)	3.89 ad (.47)	3.16 ae (.17)	0.56 be (.05)	7.46 ad (1.96)	1.32 ad (.22)	5.6 (0.6)
100 N / 1000 P	75.21 bd (32.19)	0.67 be (.14)	3.10 ae (.46)	1.00 ad (.14)	5.21 ad (1.19)	1.64 ad (.14)	3.3 (1.3)
1000 N / 10 P	5.37 af (1.41)	0.02 ag (.00)	4.87 ad (.60)	0.27 ce (.03)	6.90 ad (2.20)	0.38 bde (.12)	17.9 (0.5)
1000 N / 100 P	4.97 af (.59)	0.022 ad (.006)	5.10 ad (.25)	0.66 be (.07)	5.20 ade (.09)	0.68 be (.02)	7.7 (0.9)
1000 N / 1000 P	1.74 be (.49)	0.007 be (.002)	4.68 ad (.17)	1.14 ad (.06)	4.96 ad (1.24)	1.20 ae (.29)	4.0 (0.3)
1000 N / 10 P / 1000 Na	1.74 b (.88)	0.010b (.01)	4.49 a (.47)	0.18 c (.02)	2.26 a (1.99)	0.09 c (.08)	25.1 (1.4)