Chapter 4 Response of Chilean needle grass (*Nassella neesiana*) and phalaris (*Phalaris aquatica*) seedlings and mature plants to changes in soil phosphorus, nitrogen and soil pH

4.1. Abstract

Chilean needle grass (*Nassella neesiana* (Trin. & Rupr.) Barkworth, CNG) is commonly found growing in pasture swards with more desirable and more palatable pasture species such as *Phalaris aquatica*. CNG is known to replace more desirable species and compete for soil resources in pasture and grazing situations. This chapter describes an experiment which evaluated the relative responses of CNG and phalaris to different soil pH levels and the addition of phosphorus and nitrogen in a glasshouse environment. The application of these treatments was to establish what soil conditions favoured the growth of each of the species.

The addition of phosphorus generally increased the growth of both species. Phalaris plants were taller than CNG plants during the late seedling stages although CNG was taller by plant maturity across all soil pH treatments. Soil with neutral pH favoured both species. The plant height response of phalaris to soil phosphorus and pH was relatively larger than that of CNG plants. Overall, plant productivity was reduced across both species in acid or alkali soils without additional phosphorus.

Plants that were treated with nitrogen fertiliser were excluded from the analysis as they had symptoms of nitrogen burning and their growth was detrimentally affected.

Under glasshouse conditions, CNG was able grow more rapidly than phalaris, producing larger and taller plants, although its general trends were similar to phalaris. Although phalaris may be more responsive during early growth to certain soil management techniques, such as soil pH, the overall response of phalaris during later growth was less than that of CNG. These results suggest that CNG is likely to have a competitive advantage over phalaris in the field and that alteration of pH or soil phosphorus level is unlikely to alter that.

4.2. Introduction

Chilean needle grass (*Nassella neesiana* (Trin. & Rupr.) Barkworth, CNG) is commonly found growing in pasture populations together with more palatable species such as cocksfoot (*Dactylis glomerata*) and phalaris (*Phalaris aquatica*). The growth of CNG within the pasture sward implies that similar soil conditions are required for each of the species, however CNG is able to establish and compete against more palatable species in a range of soil types, often in paddocks that are heavily grazed or that have soils that may limit palatable grass growth. The soil nutrient requirements and optimum soil pH for CNG growth are unknown. Understanding the soil nutrient and soil pH requirements of CNG may enable manipulation of these factors to provide a competitive advantage to the more palatable beneficial grass species and be used as a tool to manage CNG infestations.

4.2.1. Effects of unpalatable grasses

CNG is considered to be an unpalatable grass when it is reproductive, which has implications for selective grazing, seedling competition and available soil nutrients.

Compared with unpalatable grasses, Moretto *et al* (1995) found that the root and leaf litter decomposition rate in palatable species was significantly faster. They also found that the palatable grasses released significantly more nitrogen (N) and phosphorus (P) from the leaf litter when compared with unpalatable grasses, yet this was not the same for the release from the roots. Thus as palatable species die, greater amounts of nutrients are released into the soil than if unpalatable species die. Making the soil more fertile for new seedling growth. Similar theories related to soil N use by native (e.g. *Nassella pulchra*) or introduced grasses (e.g. *Phalaris aquatica*) in northern coastal California have been tested by Thomsen *et al* (2006), although soil N did not appear to influence competition between the native and exotic perennial grasses in the study.

On the other hand, unpalatable grasses have a low tissue nitrogen content, thus giving their litter a high C/N ratio, which may restrict seedling growth of palatable grasses (Moretto and Distel 1997).

In glasshouse and ungrazed trials completed by Moretto and Distel (1997), the presence of unpalatable grasses did not affect the seed production of palatable grasses. This lack of effect may be due to the palatable grasses having a higher competitive ability than the unpalatable grasses in the absence of grazing.

4.2.2. Soil nutrient dynamics

Phosphorus is a constituent of compounds involved in respiration, photosynthesis, energy expenditure, cell division and growth (Cayley and Saul 2001). If phosphorus is limiting in the soil the growth of species adapted for high fertility soils may be restricted. Thus limited soil phosphorus assists species more suited to low phosphorus soils to dominate. Phosphorus is mainly measured as 'Olsen P' as milligrams of phosphorus per kilogram of soil. An Olsen P of greater than 8 is required for persistence of perennial desirable grasses and to suppress low quality grasses (Cayley and Saul 2001).

Compounds responsible for transport of substances across cell membranes, including the uptake of nutrients from soil and their movement through a plant, contain phosphorus. In the paddock, phosphorus is continually moving between the soil, plants and animals. Animals ingest some phosphorus and the remainder is returned to the soil in plant litter. The farm may then lose phosphorus in meat/milk/wool or when animals are sold off the farm (Cayley and Saul 2001). Soil nutrient availability is also affected by soil pH. This is demonstrated as phosphorus is less available to plants in soils with strongly acid or basic pH levels (GRDC *et al.* 1998).

Soil pH can indirectly affect soil nutrient availability by stimulating increased pasture growth that limits nutrient runoff and deep drainage (White *et al.* 2000). A large proportion of phosphorus is returned to the soil via dung, which can often accumulate at stock camps. Phosphorus can be adsorbed to clay to form insoluble compounds or be leached off, as in acid sandy soils, via run off and heavy rain. Phosphorus is also exported off farm via fodder (Cayley and Saul 2001). Phosphorus was therefore identified as a key element in assessing CNG nutrient requirements.

Likewise, nitrogen was selected because it is able to increase the relative growth rate of pasture grasses (Hill *et al.* 2005). Plants growing in soils that are nitrogen deficient often export carbon to their roots (Cullen *et al.* 2006) rather than growing shoots. Given that nitrogen and ammonium uptake relies on specific molecular transport systems that operate simultaneously via concentration gradients, external nitrogen has major role in plant uptake, although nitrogen uptake is also regulated by physiological status of the plant (Gastal and Durand 2000).

4.2.3. Pasture composition change

Competition between plants can be used to alter pasture composition. To take advantage of plant competition, the characteristics of both the favourable and weed plants need to be understood (Hill 1997). When weeds are germinating, it is imperative to maintain vigorous pasture. Changes in soil fertility and pH can affect the persistence of desirable pasture species such as phalaris or lucerne (Horsnell 1985; Ridley and Coventry 1992; Ridley and Windsor 1992) and can also be used to place low fertility weeds at a competitive disadvantage (Hill 1997). When applying fertiliser in a crop or when sowing pastures, fertiliser should be banded with the sown seed, rather than broadcast, to provide a direct advantage to the sown seedlings, rather than weeds between the rows (Dowling *et al.* 2000).

Grazing management can be used to reduce pasture residues and allow clover germination (Hill 1997). Deferred grazing over autumn followed by a 2 week on / 6 weeks off rotation can favour perennial grasses (e.g. *Phalaris aquatica, Lolium perenne*)(Cullen *et al.* 2006) over annual weeds in a winter rainfall region (Hill 1997). A plant population shift between legumes and grasses can also be achieved on nutrient deficient land through fertiliser application because legumes will respond more to phosphorus and sulfur while grasses will respond more to N – although timing is critical to stimulate pasture growth rather than weed growth (Taylor and Sindel 2000).

4.2.4. Fertility requirement of Chilean needle grass

Participants of a workshop discussing CNG management described CNG as having a higher fertiliser requirement for growth than serrated tussock (*Nassella trichotoma*). Yet CNG is able to infest and outcompete serrated tussock in bare patches or under the canopy of serrated tussock (Bedggood and Moerkerk 2002). Where phosphorus is added to CNG infestations, improved pasture (e.g. *Phalaris aquatica*) is said to respond better than CNG (Bedggood and Moerkerk 2002). This area of manipulating soil nutrients for CNG control has not been scientifically tested and needs further investigation.

4.2.5. Experimental aims

This experiment aimed to evaluate the relative responses of CNG plants to different soil pH levels and the addition of phosphorus and N in a glasshouse environment when compared with *Phalaris aquatica*, a palatable grass species commonly grown in Victoria where CNG is invading. The purpose of these treatments was to establish what soil conditions favoured the growth of each of the species.

4.3. Materials and methods

4.3.1. Location

This experiment was conducted in the Victorian Department of Primary Industries Frankston Centre glasshouse over winter and spring 2005. The soil used for the study was taken from a paddock in Greenvale (38°04' S, 141° 40' E) on the 21st of May 2005. The Greenvale paddock had been historically grazed by sheep, with a more recent history of horse grazing. The pasture within the paddock was primarily comprised of CNG, with sparse levels of other desirable pasture species (*Phalaris aquatica, Dactylis glomerata and Lolium perenne*). Plants in the glasshouse trial were irrigated with 50 mm of water (crosschecked with rain gauges) every Monday, Wednesday and Friday, via overhead sprays. Glasshouse temperatures were kept between 10-24°C.

4.3.2. Design and treatments

The experiment consisted of 3 levels of soil phosphorus addition (none, half limiting 20 μg P/g soil, non limiting 40 μg P/g soil), 3 levels of soil nitrogen addition (none, 46 kg N, 92 kg N) and 3 soil pH levels (approximately pH 5, 7 and 9) in a two species factorial design replicated 3 times.

Pots were positioned in a randomised block design across three benches with pots re-randomised within each block at each measurement event (Figure 4.1). Seedlings of both species were grown from seed in heated germination trays with seedling starter mix (17th May 2005, Figure 4.2). CNG seedlings were grown from CNG panicle seed harvested at Greenvale in December 2003, whilst a persistent and winter active cultivar of phalaris that has vigorous seedlings, 'Holdfast,' was used to grow the phalaris seedlings (Stephen Pasture Seeds)(AWI 2004). Two seedlings of each species were transplanted into their respective pots on the 13th June 2005 (Figure 4.3). The experimental pots consisted of PVC pipe (90 mm diameter x 350 mm deep) with fly mesh blocking the base, and a saucer to hold drained water.

4.3.3. Pre experimental soil extraction

A front end loader was used to remove the top 25 mm of soil from the Greenvale paddock prior to extracting the following 150 mm of soil for use in the trial (Figure 4.4). Soil was transported to the Department of Primary Industries Frankston Centre for steam sterilisation at 85°C for 3 hours. Sterilised soil was sieved (10 mm screen) to remove large soil peds and rocks.

4.3.4. Pre experimental soil analysis

The soil from the Greenvale paddock (tested prior to soil extraction and addition of ameliorants) was a grey brown fine to very fine sandy loam (Olsen P 6.1 mg/kg, pH ($CaCl_2$) 4.7 – FarmRight Technical Services Kyabram).

Figure 4.1 CNG and phalaris plants in glasshouse, randomised within each bench.

Figure 4.2 CNG and phalaris seedlings growing in germination trays.

Figure 4.3 Transplanting seedlings into PVC pipe pots.

Figure 4.4 Extracting soil from Greenvale paddock

4.3.5. pH buffer curve

The pH buffering capacity of the soil was determined at the Institute of Land and Food Resources laboratory, The University of Melbourne, Parkville. Appropriate concentrations of NaOH (0.5 M) and HCl (0.5 M) (Table 4.1) along with 12.5 ml of 0.1M KCl, were added to two replicates of seven 30 ml plastic vials, each with 5 g of paddock soil (100 mm depth). The soil solutions were shaken end over end for 17 hours prior to determining their pH using a pH probe (ILFR 2001). A trend line for the soil pH change was then established from the data points (Figure 4.5, $R^2 = 0.99$).

Table 4.1 Amounts of alkali or acid required for the pH buffer curve.

Tube number	ml of 0.5 M HCl	ml of 0.5 M NaOH
1	0.6	0
2	0.4	0
3	0.2	0
4	0	0
5	0	0.2
6	0	0.4
7	0	0.6

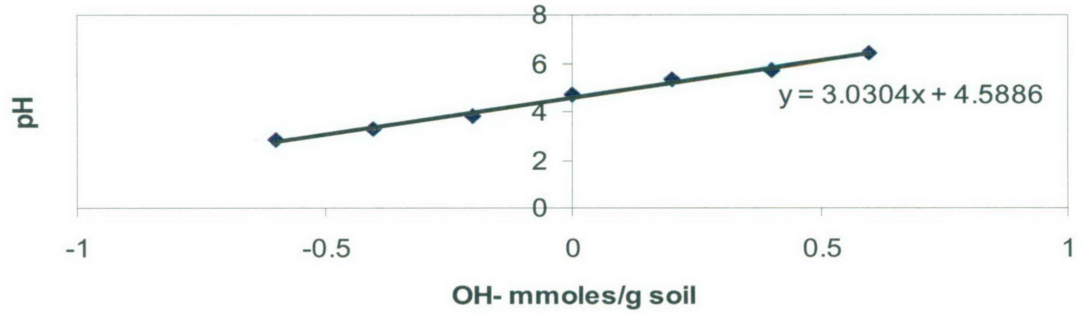

Figure 4.5 pH buffering curve for the Greenvale soil.

4.3.6. Phosphorus adsorption isotherm

The phosphorus buffering capacity of the paddock soil was determined at the Institute of Land and Food Resources laboratory, Parkville. Four 30 ml phosphorus solutions, of differing concentrations (Table 4.2), were added to three replicates of 2.5 g soil (100 mm depth) samples in plastic vials. The soil solutions were shaken end over end for 1 hour at room temperature and centrifuged for 10 minutes. Following centrifugation, samples were filtered and the resulting aliquots captured into separate vials (ILFR 2001). To these samples, 4 ml of phosmolybdate (colour reagent) was added and the solution made up to 30 ml using deionised water. After 20 minutes the adsorbance of each sample was read using a UV-visible spectrophotometer at 883 nano meter (nm) wavelength (Figure 4.6).

Table 4.2 Concentrations of KH_2PO_4 solution added to determine the P adsorption isotherm.

	Solution 1	**Solution 2**	**Solution 3**	**Solution 4**	**Solution 5**
µg P/ml	0.08	0.8	2.1	4.2	8.3

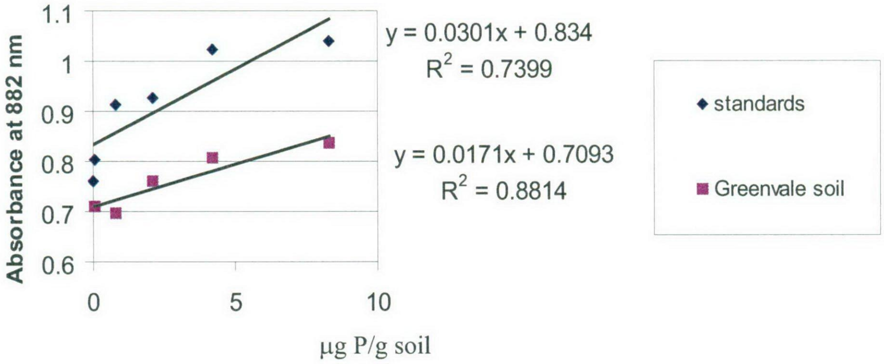

Figure 4.6 P adsorption isotherm of Greenvale soil and standard solutions.

4.3.7. *Application of soil treatments*

Initial soil ameliorants were added to the soils prior to transplanting the seedlings. Nitrogen was applied as a split dressing and was determined by the pre-experimental soil analysis in relation to the buffering capacity of the measured soil factors. Nitrogen addition treatments were calculated to be equivalent to 200 kg/ha (92 kgN/ha) and 100 kg/ha (46 kgN/ha) Urea ($CO(NH_2)_2$). Nitrogen was applied by spray solution (138 g urea/10 L water) at rate of 50 ml per pot, and a follow up spray for 200 kg/ha pots on 25th July 2005 at a rate of 50 ml per pot. Follow up nitrogen applications for the 92 kg N /ha treatment occurred after transplanting.

Phosphorus addition treatments (KH_2PO_4) were applied by spray solution (6g KH_2PO_4/L water, Figure 4.7) at the rate of 100 ml per pot for non limiting plant growth (40 μg P/g soil) or 50 ml per pot for half limiting plant growth (20 μg P/g soil).

In order to adjust soil pH, lime was added and mixed through 40 kg batches of soil using a cement mixer prior to potting up. Liming rates were equivalent to 7.9 t/ha (pH 7) and 14.8 t/ha (pH 9) of agricultural grade lime (79% neutralising value).

Additional soil nutrients were sprayed onto the soil as a solution (300 ml/pot) to eliminate other nutrient deficiencies that could affect plant growth (Table 4.3). Nutrient solution was applied on the 12th July (300 ml per pot) and 25th July (50 ml per pot) to all pots, and re-applied to mature phase plants on the 22nd August (50 ml per pot) to ensure adequate nutrition for continued growth.

Table 4.3 Additional nutrient solutions sprayed onto soil.

Element	Solution	Concentration
Fe	Iron sulfate	0.1 mM
Mo	Sodium molybdate	0.0007 mM
Mn	Manganous sulfate	0.01 mM
Cu	Copper sulfate	0.001 mM
Ca	Calcium carbonate	5.0 mM
Mg	Magnesium sulfate	2.0 mM
K	Potassium chloride	20 mM
S	Sulfur as sulphate	n/a

4.3.8. Sampling

There were two destructive harvests within the experiment. The seedling phase ended at day 40 (22nd August 2005) when plants began to tiller, whilst mature plants were harvested during seed production at day 162 (22nd December 2005). Plant heights were measured weekly (seedlings) or fortnightly (mature plants) prior to harvests (See appendices Table A4.1). At harvest, plant samples were separated into root and shoot sections, with the shoot further divided into leaf and stem. Total leaf area was measured using a Paton Electroplan Electric Planimeter whilst root length (root depth) was measured using a ruler for seedlings as fresh samples. All plant sections were then dried at 100°C for 7 days to determine root dry weight and shoot dry weight. Root length could not be measured for plants that grew to maturity as roots were entangled at the base of the pots due to the extended growth period. Results are generally presented on a per pot basis.

Figure 4.7 Spraying nutrient solution into plant pots

4.3.9. Statistical analysis for seedling measurements

After each measurement had been appropriately transformed, the data were analysed by analyses of variance with the structure presented in Table 4.4. Because the treatments with nitrogen added had symptoms of nitrogen burning (in particular their growth was less than corresponding treatments without nitrogen added), the treatments were analysed with a species by phosphorus by pH factorial structure within treatments that received no added nitrogen. The treatments with nitrogen applied were bulked together as a nuisance term with 35 degrees of freedom so that the residual variation could be estimated from all pots in the experiment. As preliminary analyses indicated that there was little difference between treatments with half limiting P and non limiting P, the effects of P were divided into the orthogonal components of:

(1) no P added vs half limiting or non limiting P , and

(2) half limiting P vs non limiting P.

As there was also little evidence of species by pH interactions or species by P by pH interactions, within no nitrogen added, the results are presented as:

(1) table of Species by no P added vs P added combinations, and

(2) pH by No P added vs P added combinations.

4.3.10. Statistical analysis for mature plant measurements

Mature plant data were analysed the same as for seedlings except that heights recorded earlier were analysed using analyses of variance similar (Table 4.5) in structure to those used in the seedling stage of the experiment. As preliminary analyses indicated that there was little difference between treatments with half limiting P compared with corresponding treatments with non limiting P, the effects of P were divided into the orthogonal components of:

(1) no P added vs half limiting or non limiting P added, and

(2) half limiting P vs non limiting P.

Since there was also little evidence of Species by pH interactions or Species by P by pH interactions, the results are presented as graphs and tables of:

(1) species by No P added vs P added combinations, and

(2) pH by No P added vs P added combinations.

Table 4.4 Sources of variation in the analyses of variance for measurements in the seedling growth phase component of the study.

Sources of variation	**DF**
Reps	2
N_0 vs N added	1
P within N_0	2
Effect of any P added within N_0	1
P 20 vs P 40 within N_0	1
pH within N_0	2
Species.P within N_0	2
Species.effect of any P added within N_0	1
Species. P 20 vs P 40 within N_0	1
Species.pH within N_0	2
P.pH within N_0	4
Effect of any P added.pH within N_0	2
P 20 vs P 40.pH within N_0	2
Species.P.pH within N_0	4
Species.Effect of any P added.pH within N_0	2
Species.P 20 vs P 40.pH within N_0	2
Treatment combinations within either N 20 or N 40	35
Residual	106
Total	161

Table 4.5 Sources of variation in the analyses of variance for measurements in the mature growth phase component of the study (excluding heights up to day 96 that were analysed using analyses of variance with similar structure to that used in the seedling phase).

Sources of variation	DF
Rep	2
Species	1
P	2
Effect of any P added	1
P 20 vs P 40	1
pH	2
Species.P	2
Species.effect of any P added	1
Species. P 20 vs P 40	1
Species.pH	2
P.pH	4
Effect of any P added.pH	2
P 20 vs P 40.pH	2
Species.P.pH	4
Species.Effect of any P added.pH	2
Species.P 20 vs P 40.pH	2
Residual	34
Total	53

4.4. Results

Plants that were treated with nitrogen fertiliser have been excluded from the analysis, as they had symptoms of nitrogen burning and their growth was detrimentally affected.

4.4.1. Seedling response to phosphorus

Phalaris seedlings, averaged across both phosphorus treatments, had significantly heavier shoots (Figure 4.8) and roots (Figure 4.9) with significantly more leaf area (Figure 4.10) than CNG seedlings (all $P < 0.001$). The root to shoot ratio of the seedlings was not significantly different for the two species. The addition of phosphorus, irrespective of species, significantly increased seedling shoot and root weight, leaf area, root length (Figure 4.11)(all $P < 0.001$) and root to shoot ratio ($P = 0.004$)(See appendices Table A4.2). The plant growth increase, in response to the addition of soil phosphorus was greater in phalaris than CNG. This result was seen for phalaris and CNG, respectively, in terms of root (+0.26g:+0.07g) and shoot weight (+0.23g:+0.08g, both $P = 0.001$), leaf area (+71cm^2:+15cm^2) and root length (+18.5cm:+3.2cm, both $P < 0.001$).

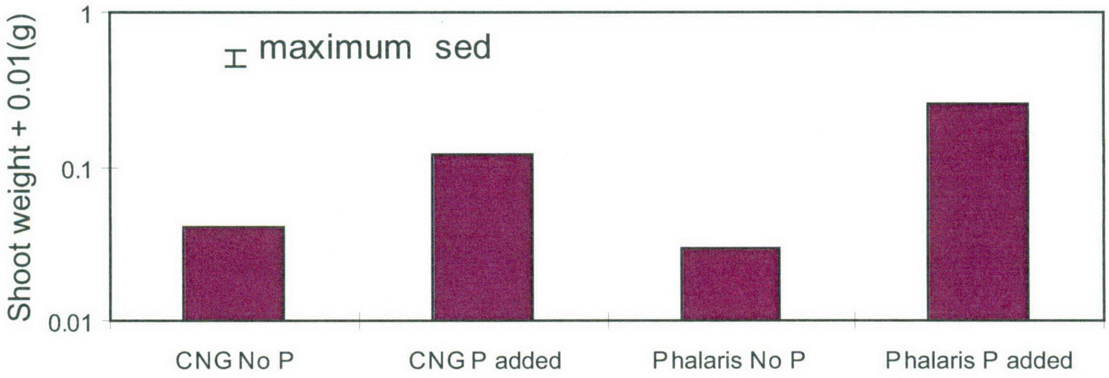

Figure 4.8 Effect of phosphorus for each species on seedling shoot weight at harvest. Note: Maximum s.e.d. is calculated on log shoot wt + 0.025 scale.

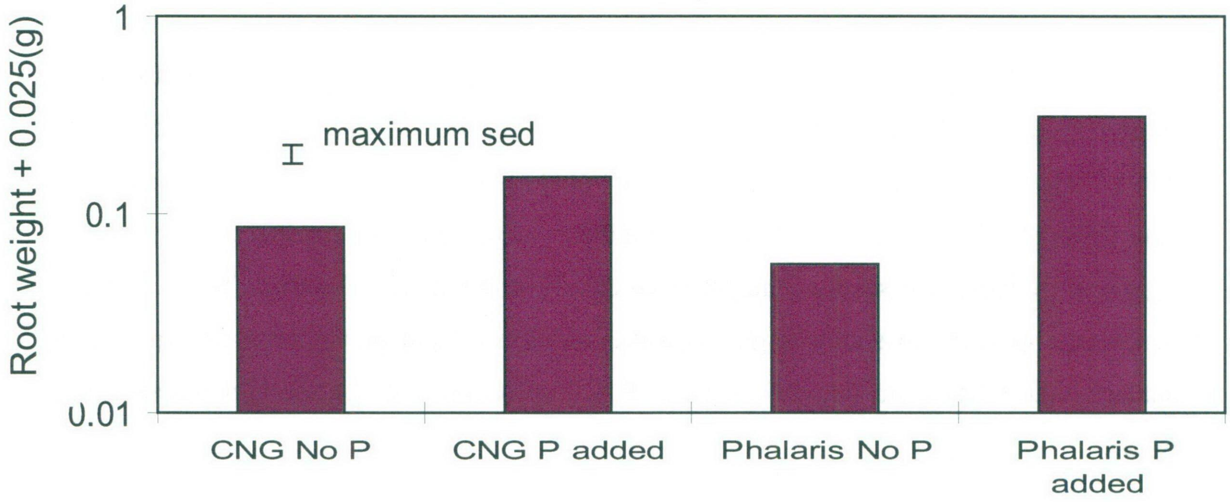

Figure 4.9 Effect of phosphorus for each species on seedling root weight at harvest. Note: Maximum s.e.d. is calculated on log root wt + 0.025 scale.

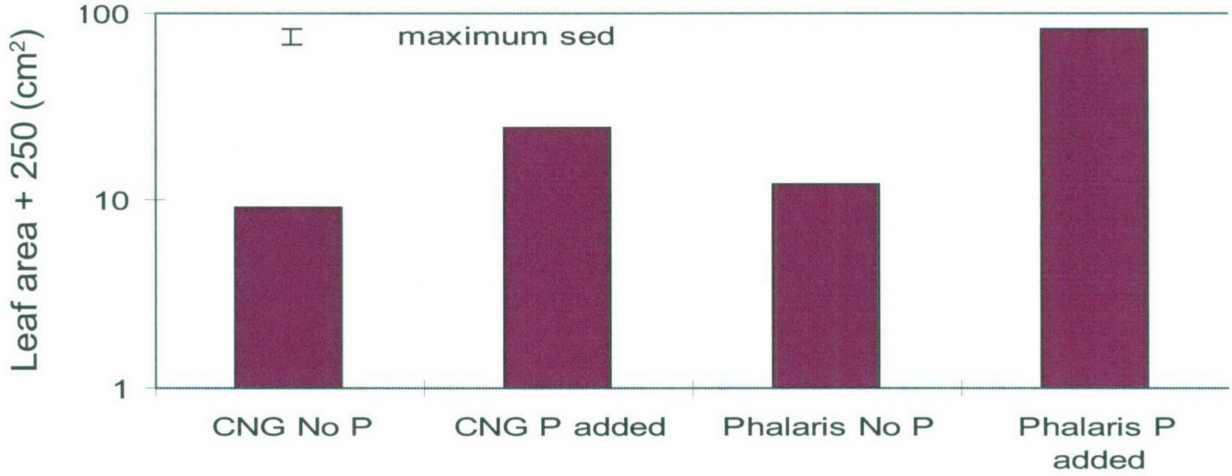

Figure 4.10 Effect of phosphorus for each species on seedling leaf area at harvest. Note: Maximum s.e.d. is calculated on leaf area + 0.025 scale.

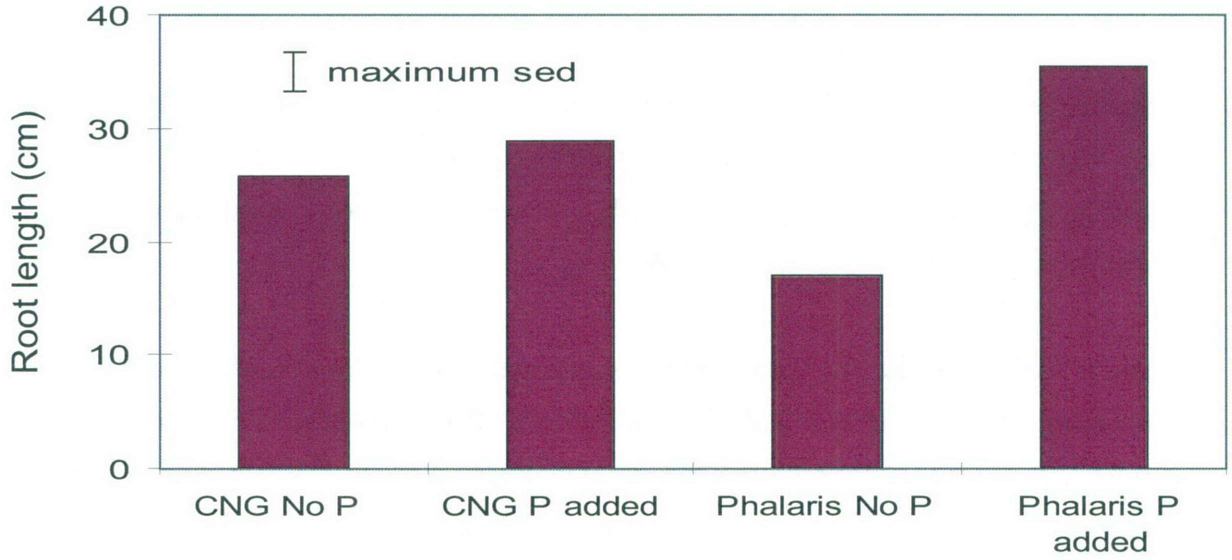

Figure 4.11 Effect of phosphorus for each species on seedling root length at harvest.

Phalaris seedlings, averaged across both phosphorus added treatments, had significantly shorter seedlings (Figure 4.12) than CNG during early growth (days 5 and 12, both P < 0.001). However, from approximately day 17 phalaris seedlings with P added grew taller than CNG seedlings with P added. The addition of phosphorus, irrespective of species, significantly increased seedling height from day 12 onwards (P < 0.001). The actual and percentage increase in shoot height with the addition of soil phosphorus was greater in phalaris than CNG from day 19 onwards (P < 0.001)(Figure 4.12)(See appendices Table A4.3).

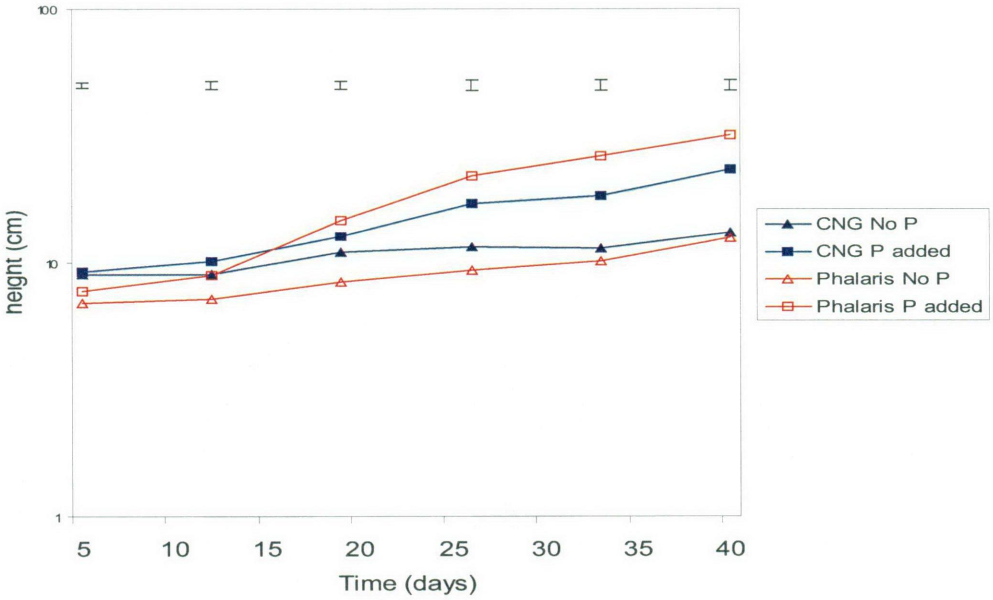

Figure 4.12 Effect of phosphorus on seedling height over time. Error bars represent maximum standard error of difference.

4.4.2. Seedling response to soil pH

Together, seedlings of CNG and phalaris in the pH 7 treatment had significantly more leaf area than seedlings in the pH 5 or pH 9 treatments (P = 0.033). Soil pH did not significantly affect shoot weight, root length, root weight, or root to shoot ratio though growth was generally optimal at pH 7 (Table 4.6).

Phalaris seedlings in the pH 7 treatment had significantly greater leaf area than those in pH 9 treatment (P = 0.033)(Figure 4.13). Soil pH did not affect shoot weight, root length, root weight or the root to shoot ratio in either species (Figure 4.14, Figure 4.15, Figure 4.16). There was no interaction between species or soil pH for these factors (See appendices

Table A4.4).Overall the seedling height response of CNG and phalaris, to changes in soil pH was not significantly different (Figure 4.17)(See appendices Table A4.5).

4.4.3. Interaction between soil pH and phosphorus on seedling growth

There was no significant interaction between the addition of phosphorus fertiliser and soil pH in terms of shoot or root weight, root length or root to shoot ratio for both species (Table 4.7), although a general trend was observed with the addition of phosphorus and soil pH on seedling productivity (interpreted from measured growth factors)(Figure 4.18). Seedlings in soil without added phosphorus grew better at pH 7 than those at pH 5 or pH 9 (See appendices Table A4.6).

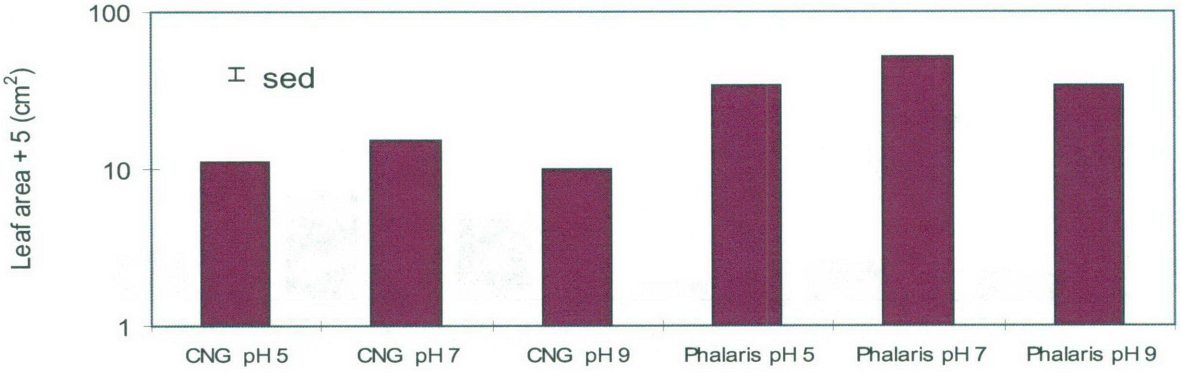

Figure 4.13 Effect of soil pH for each species on seedling leaf area at harvest

Note: Maximum s.e.d. is calculated on log leaf area + 5 scale.

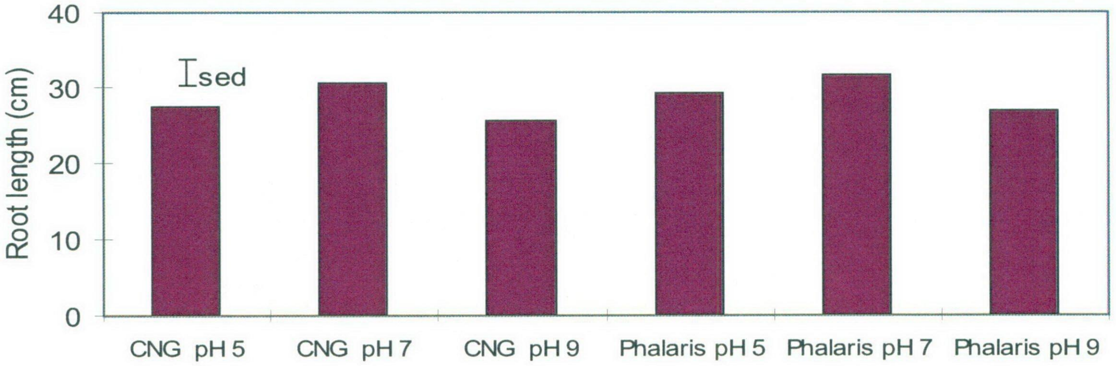

Figure 4.14 Effect of soil pH for each species on seedling root length at harvest.

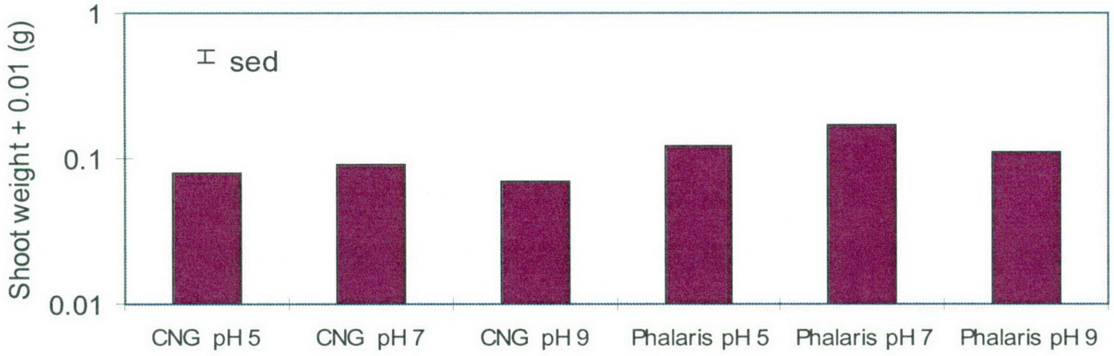

Figure 4.15 Effect of pH for each species on seedling shoot weight at harvest. Note: Maximum s.e.d. is calculated on log shoot wt + 0.01 scale.

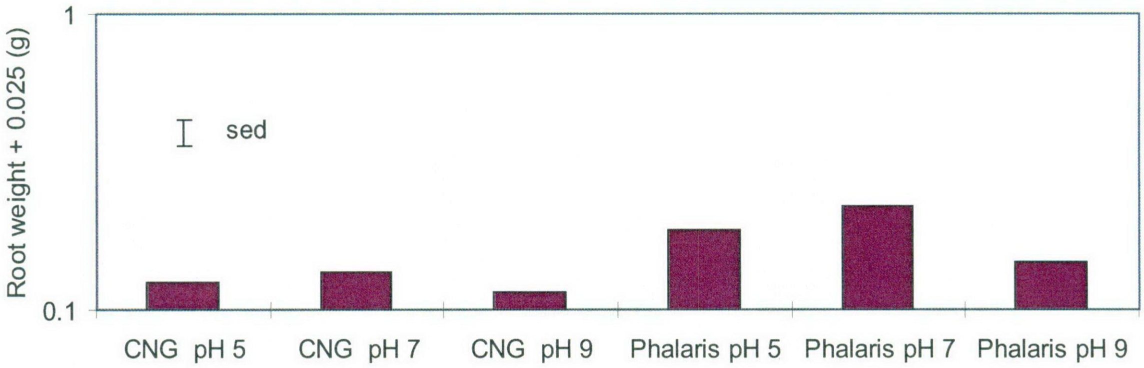

Figure 4.16 Effect of pH for each species on seedling root weight at harvest. Note: Maximum s.e.d. is calculated on log root wt + 0.025 scale.

Table 4.6 Effect of soil pH across both species (CNG and phalaris) on seedling shoot weight, leaf area, root length, root weight and root to shoot ratio at harvest (No nitrogen treatments, s.e.d. standard error of difference).

Measurement	**pH 5**	**pH 7**	**pH 9**	**s.e.d.**	**P Value**
Shoot Wt - Log (x + 0.01)	-1.01	-0.91	-1.05	0.067	0.097
Backtransformed (g)	0.09	0.11	0.08	-	-
Leaf area - Log (x + 0.025)	1.40	1.53	1.39	0.058	**0.033**
Backtransformed (cm^2)	20	29	19	-	-
Root Length (cm)	28.3	31.0	26.2	2.51	0.163
Root Wt - Log (x + 0.025)	-0.81	-0.76	-0.89	0.060	0.106
Backtransformed (g)	0.13	0.15	0.10	-	-
Root:Shoot – Log	0.18	0.11	0.11	0.072	0.592
Backtransformed	1.5	1.3	1.3	-	-

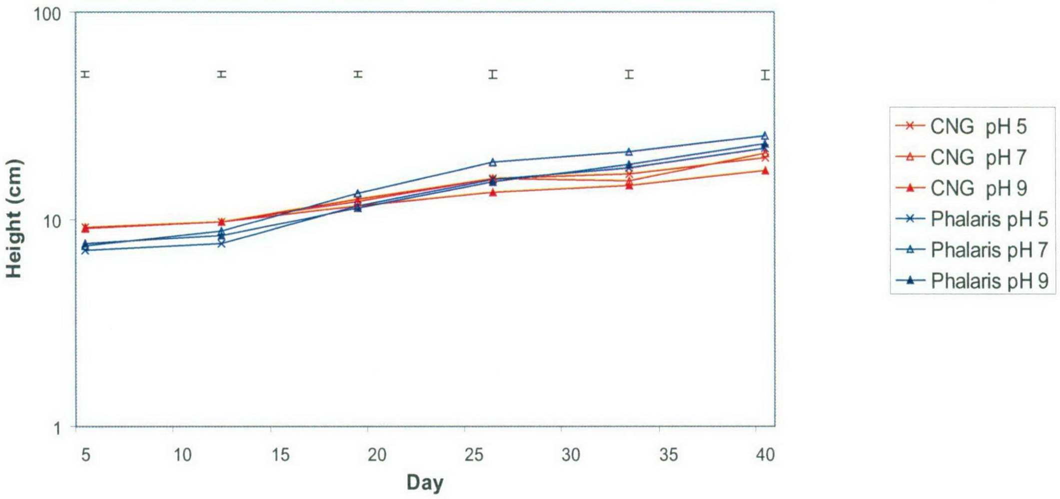

Figure 4.17 Effect of soil pH and species (CNG and phalaris) on seedling height. Error bars denote standard errors of difference. Note: Maximum s.e.d. is calculated on log height scale.

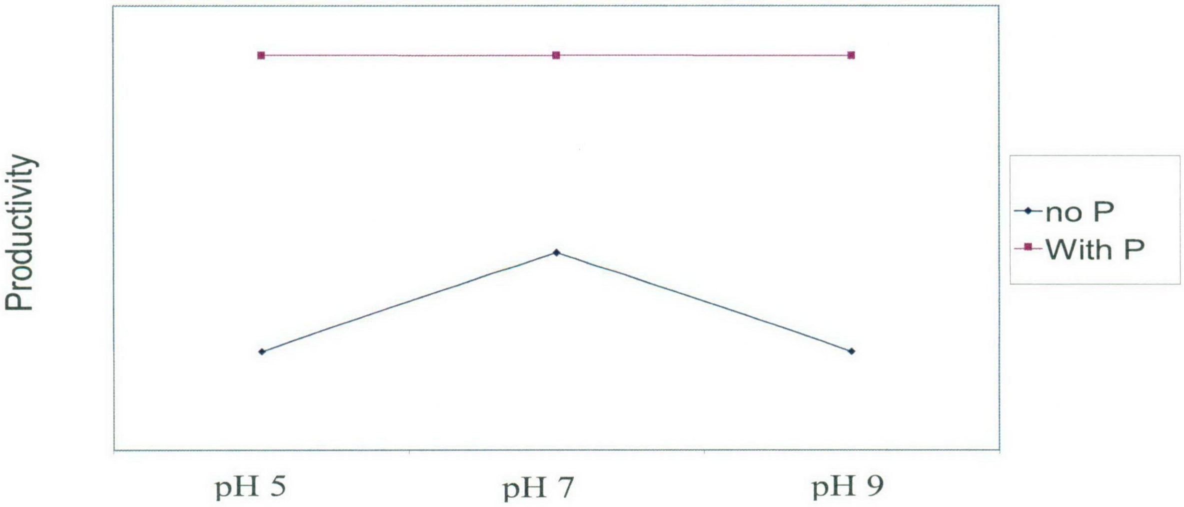

Figure 4.18 Schematic average effect of soil pH and phosphorus on overall seedling productivity (interpreted from measured growth factors).

Table 4.7 Effect of phosphorus (P added vs no P added) at different soil pH levels on combined (average of CNG and phalaris) seedling shoot weight, leaf area, root length, root weight and root to shoot ratio at harvest (No nitrogen treatments).

	Effect of phosphorus fertiliser										**P-Values**	
	pH 5			**pH 7**			**pH 9**					
	No P	P Added	Difference	No P	P Added	Difference	No P	P Added	Difference	s.e. of difference (*within pH level*)	pH main effect	Presence of P fertiliser by pH Interaction
Shoot Wt												
Log (x +0.01)	-1.57	-0.74	0.83	-1.26	-0.73	0.53	-1.53	-0.81	0.72	0.095	0.097	0.101
Backtransformed (g)	0.03	0.17	-	0.05	0.17	-	0.02	0.15	-	-	-	
Leaf area												
Log (x + 5)	0.94	1.63	0.68	1.16	1.71	0.55	0.95	1.61	0.66	0.081	**0.033**	0.647
Backtransformed (cm^2)	4	37	-	10	46	-	5	35	-	-	-	
Root Length (cm)	18.5	33.2	14.7	26.6	33.2	6.6	18.7	30.0	11.3	3.75	0.163	0.312
Root Wt												
Log (x + 0.025)	-1.19	-0.63	0.57	-1.05	-0.62	0.43	-1.21	-0.73	0.48	0.085	0.106	0.554
Backtransformed (g)	0.04	0.21	-	0.09	0.22	-	0.04	0.16	-	-	-	
Root:Shoot (Log)	0.36	0.08	-0.28	0.13	0.10	-0.04	0.26	0.04	-0.22	0.102	0.592	0.246
Backtransformed	2.3	1.2	-	1.4	1.2	-	1.8	1.1	-	-	-	

4.4.4. *Mature plant response to phosphorus*

By the time of the second harvest, at plant maturity, phalaris plants averaged across both soil phosphorus treatments (no P, P added), had significantly lighter shoots ($P < 0.001$)(Figure 4.19), significantly heavier roots ($P = 0.001$)(Figure 4.20), and a significantly higher root to shoot ratio than CNG plants (see appendices Table A4.7). Plants in the phosphorus addition treatment, averaged across both species, had significantly heavier shoot weight ($P = 0.027$) at harvest than those where no P was added. The root to shoot ratio of phalaris increased significantly more than CNG in response to the addition of soil phosphorus ($P = 0.020$).

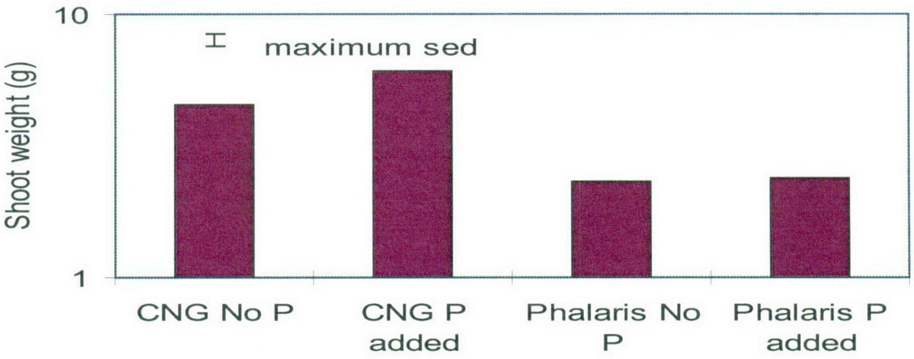

Figure 4.19 Effect of phosphorus application on mature plant shoot weight.

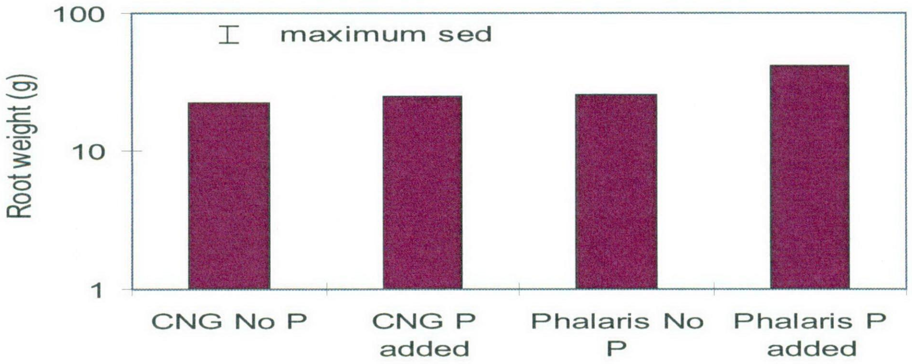

Figure 4.20 Effect of phosphorus application on mature plant root weight.

Phalaris plants, averaged across both soil phosphorus treatments (no P, P added), were significantly taller then CNG plants during early growth (generally $P < 0.001$) yet generally significantly shorter than CNG from day 75 onwards (all $P < 0.001$, see appendices Table A4.8). The addition of phosphorus, across both species, significantly increased plant height from day 12 to day 75 (generally $P < 0.001$) but by the end of the experiment had little effect (Figure 4.21).

With the addition of soil phosphorus, phalaris grew significantly taller than CNG from day 12 to day 54 however from day 75 onwards, CNG with added P grew taller than phalaris ($P < 0.05$) (Figure 4.21).

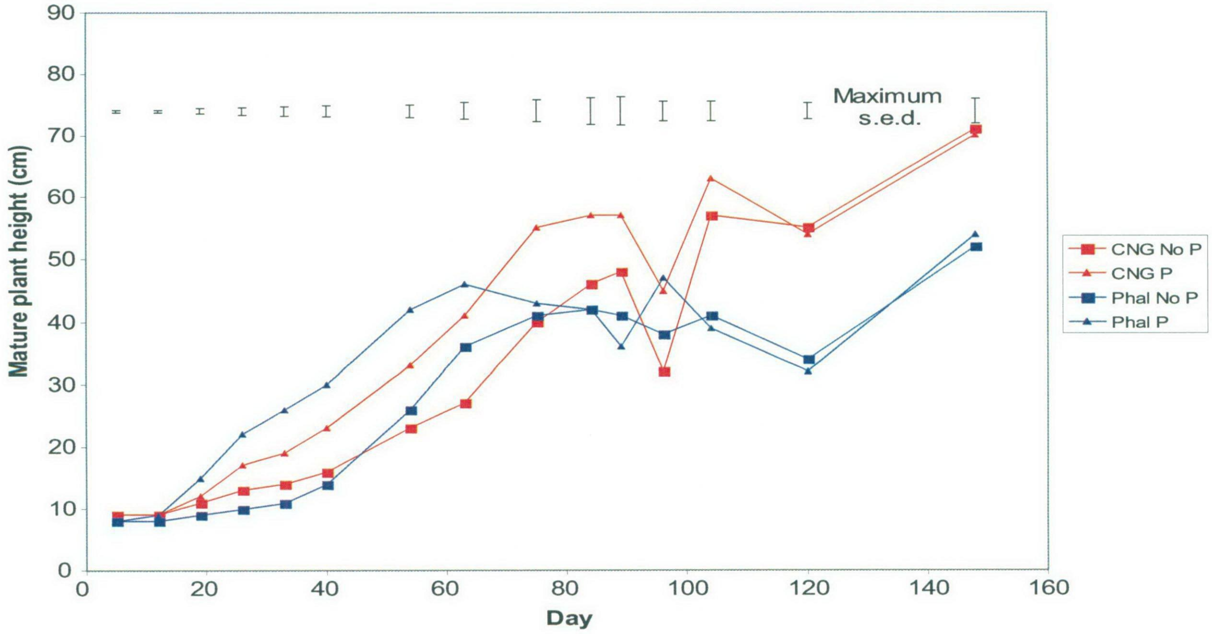

Figure 4.21 Effect of soil phosphorus on mature plant height

4.4.5. Mature plant response to soil pH

Across both species, mature plants in the pH 5 treatment had significantly lighter shoots ($P < 0.01$)(Figure 4.22) and leaf area ($P = 0.003$)(Figure 4.24) than the other pH treatments, whereas root weight was unaffected by pH (Figure 4.24). The increased shoot weight response of phalaris plants to the pH 7 and pH 9 treatments was significantly greater than CNG ($P = 0.024$)(See appendices Table A4.9).

At pH 5, both species generally grew significantly taller than plants at pH 7 or pH 9 up to day 26 (Figure 4.25). At certain times, phalaris plant height responded more favourably to the pH 7 or 9 treatments, whereas CNG plants responded more favourably to the pH 5 treatment. By harvest, plants of both species in the pH 7 or 9 treatments were taller than those in the pH 5 treatments. Although generally shorter over the first 60 days of growth, CNG plants generally grew taller than phalaris plants by harvest (Figure 4.25)(See appendices Table A4.10).

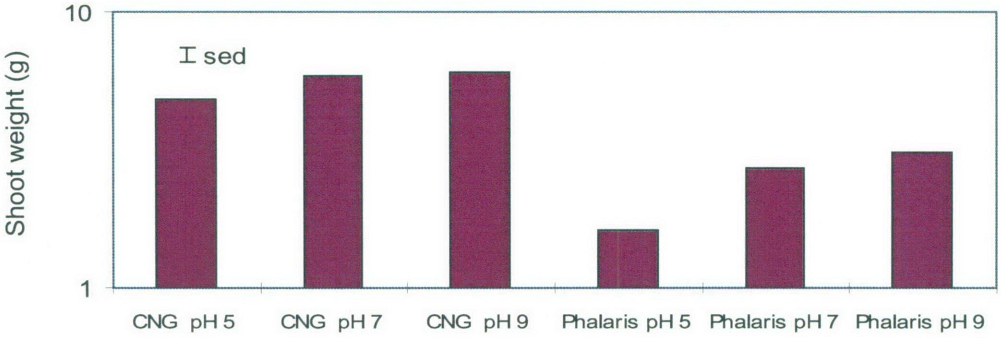

Figure 4.22 Effect of soil pH on mature plant shoot weight at harvest in each species.

Note: Maximum s.e.d. is calculated on log shoot wt scale.

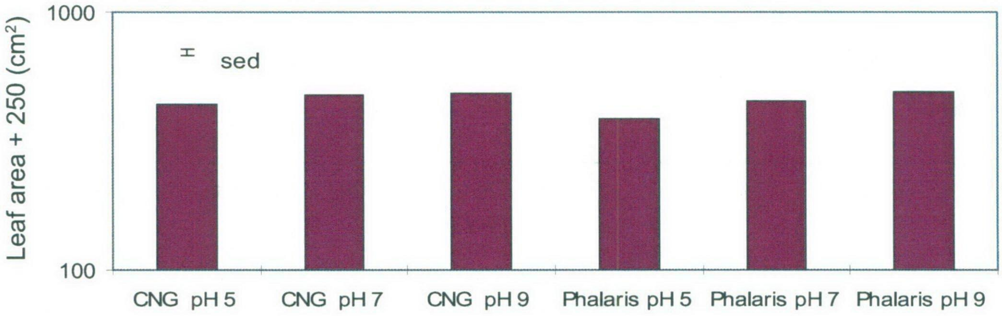

Figure 4.23 Effect of soil pH on mature plant leaf area at harvest in each species.

Note: Maximum s.e.d. is calculated on log leaf area + 250 scale.

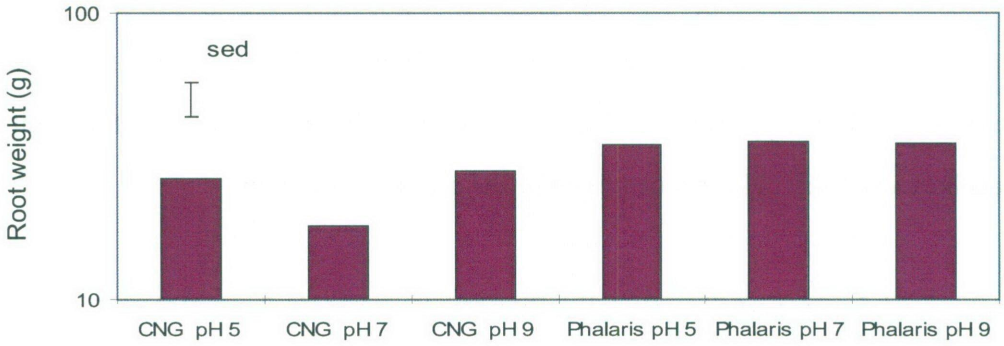

Figure 4.24 Effect of soil pH on mature plant root weight at harvest in each species.

Note: Maximum s.e.d. is calculated on log root wt scale.

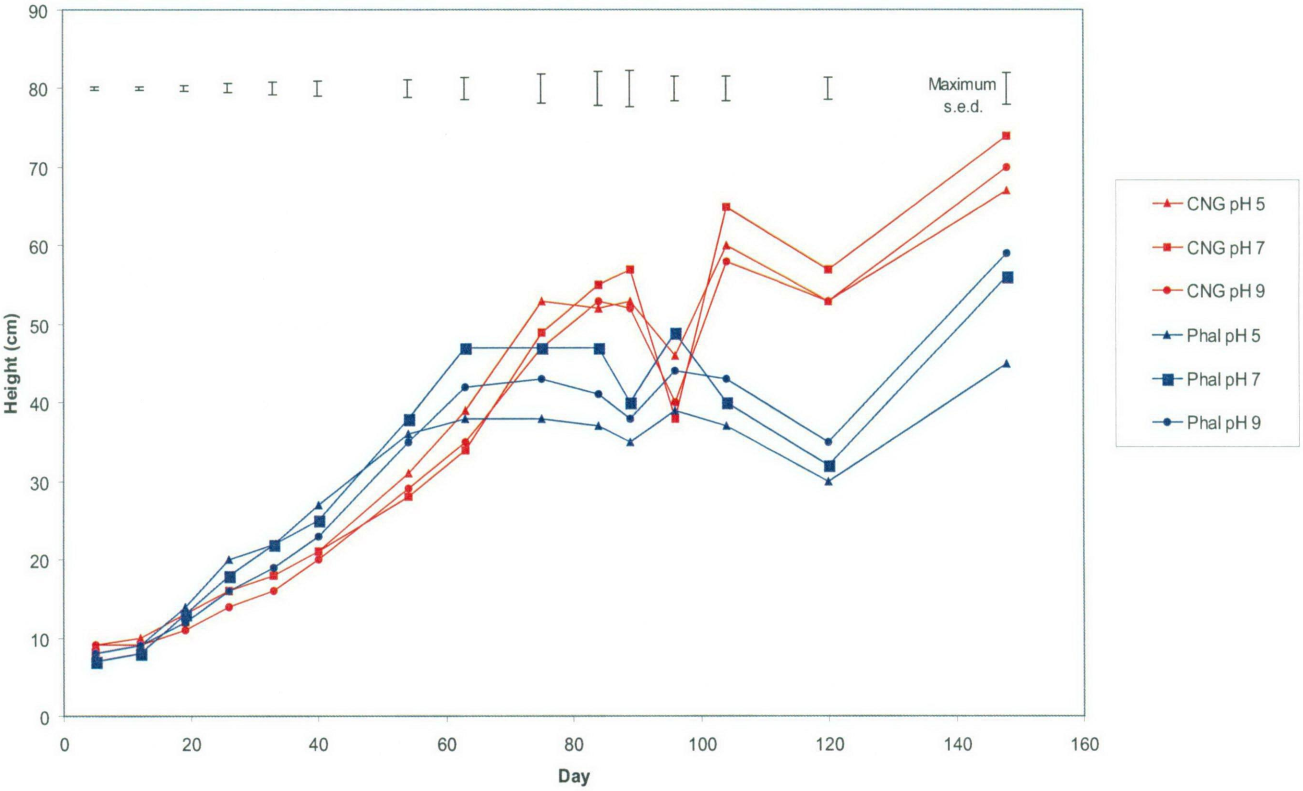

Figure 4.25 Effect of soil pH on mature plant height

4.4.6. *Mature plant growth interaction (soil pH and phosphorus)*

The response of shoot weight and leaf area to phosphorus addition, across both species, was significantly larger at high pH than neutral pH (Table 4.8). There was no significant interaction between phosphorus and soil pH for root weight or the root to shoot ratio.

Plants at pH 5 were significantly taller than plants in the pH 7 or pH 9 soil during the early growth stages in response to phosphorus. At the end CNG plants were taller than phalaris plants (Figure 4.26)(See appendices Table A4.11).

Table 4.8 Effect of phosphorus (P Added vs No P Added) at different soil pH levels on mature plant shoot weight, leaf area, root weight and root to shoot ratio at harvest (No nitrogen treatments).

Measurement	**Effect of P Fertiliser**										**P-Values**
	pH 5			**pH 7**			**pH 9**			***s.e. of effect (within pH level)***	Presence of P fertiliser by pH Interaction
	No P	P Added	Difference	No P	P Added	Difference	No P	P Added	Difference		
Shoot Wt – Log (x)	0.44	0.44	-0.01	0.60	0.60	-0.0	0.48	0.71	0.23	0.055	**0.0056**
Backtransformed (g)	2.8	2.7	(-2%)	4.0	4.0	(-1%)	3.0	5.1	(+70%)	-	
Leaf area – Log (x + 250)	2.63	2.61	-0.02	2.69	2.66	-.03	2.64	2.71	0.07	0.030	**0.045**
Backtransformed (cm2)	172	157	-	236	203	-	186	265	-	-	
Root Length – Log (x)	1.43	1.51	0.08	1.37	1.42	0.05	1.32	1.59	0.27	0.123	0.385
Backtransformed (cm)	26.9	32.1	(+19%)	23.7	26.3	(+11%)	20.7	38.5	(+86%)	-	
Root:Shoot (Log)	0.99	1.07	0.08	0.77	0.82	0.05	0.77	0.88	0.11	0.118	0.941
Backtransformed	9.8	11.8	(+21%)	5.9	6.6	(+12%)	5.9	7.5	(+28%)	-	

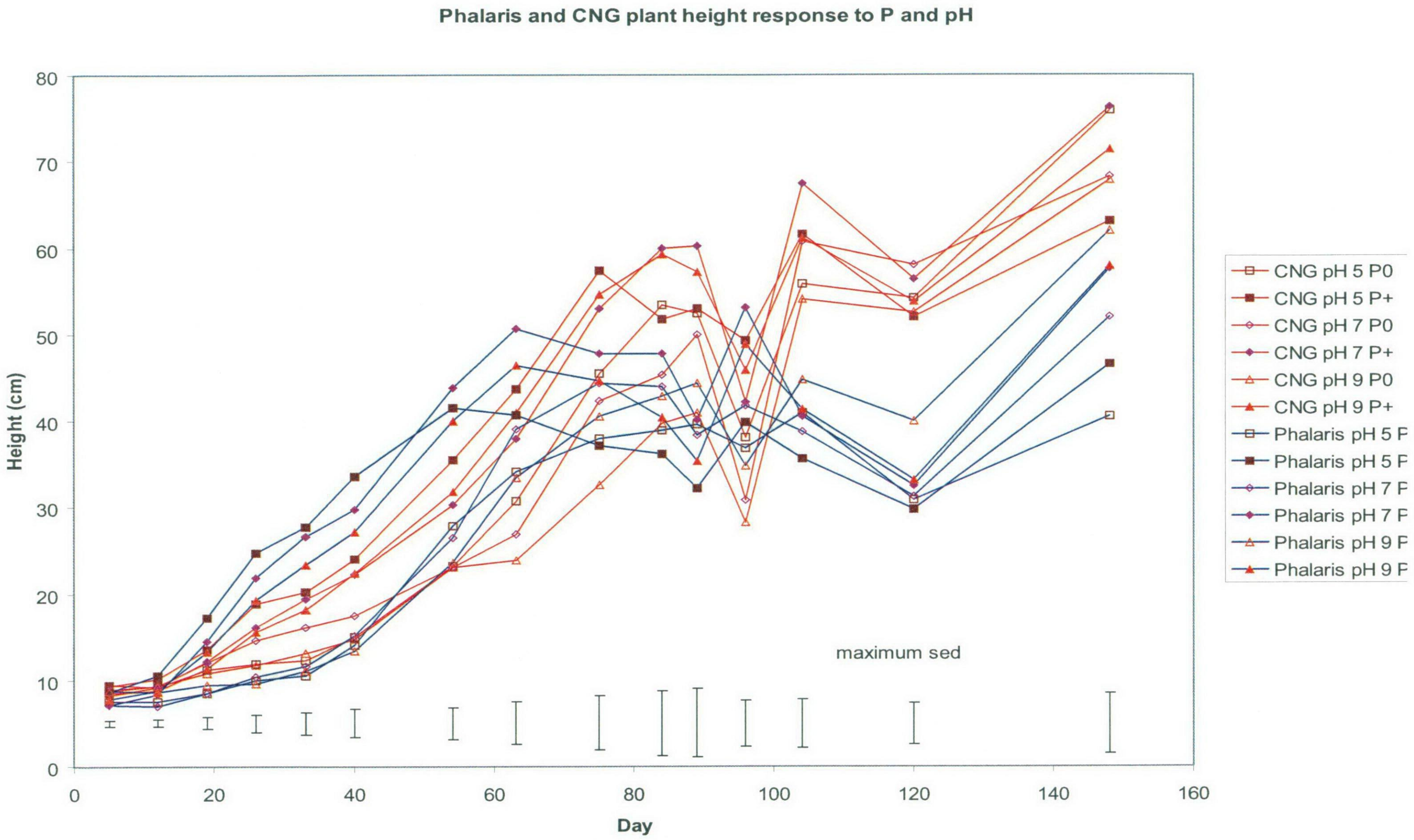

Figure 4.26 Effect of soil pH and soil phosphorus on mature plant heights of CNG and phalaris.

4.5. Discussion

4.5.1. Differences between species (irrespective of soil phosphorus additions or pH modification)

Phalaris is considered a more desirable pasture grass in grazing systems when compared with other less palatable grasses (Bourdot 1988; DNRE 2002; DPI 2000).

The height of the two species varied depending on the stage of growth. Young CNG seedlings are considered to be very vigorous. They are known to establish under the canopy of other plants as well as in open areas, and often take over from serrated tussock infestations due to bare patches created by herbicide (Bedggood and Moerkerk 2002; Bourdot 1988; Slay 2002). CNG seedlings in this trial were observed to be taller than phalaris up to day 12 after which phalaris grew taller. A switch in plant height, where CNG once again outgrew phalaris, occurred around day 63. The point at which CNG growth increased significantly could be attributed to the CNG plants becoming reproductive and vigorously producing erect stems for seed production, whereas the majority of phalaris plants did not produce seed. Whether this result is due to night temperatures in the glasshouse being too warm for vernalisation was not tested. This phenological growth difference could be an advantage to CNG in not only having taller plants but also in terms of maximising seed production.

This trial has confirmed that very young CNG seedlings are more responsive than phalaris, in relation to seedling height, and as well CNG was taller during seed production.

Based on plant height and competition for light, this finding supports observations that CNG seedlings are vigorous and able to establish and successfully compete against improved pasture species during early seedling growth, and as mature plants during seed production. However phalaris was taller during most of the vegetative stage of growth, and so does offer potential as a competitor for CNG during this period.

This trial only tested one variety of phalaris, ‘Holdfast’, which is general purpose cultivar known for its excellent seedling vigour and yield potential (AWI 2004; Reed 1999). But it is possible that other varieties may perform slightly differently to Holdfast.

4.5.2. Species response to added phosphorus

Phosphorus is a constituent of compounds involved in respiration, photosynthesis, energy expenditure, cell division and growth in plants (Cayley and Saul 2001). When phosphorus is added to soils, nutrient uptake and transport from the soil can be enhanced, and phosphorus compounds are responsible for transport of substances across cell membranes (Cayley and Saul 2001).

The addition of phosphorus (both the half limiting and the non limiting treatment) significantly increased the growth of both species as seedlings and mature plants in this phosphorus deficient soil. Initially, the response of phalaris seedlings to phosphorus was greater than that of CNG seedlings, although as the trial progressed, mature CNG plants ultimately tended to respond more to phosphorus than mature phalaris plants. Given that the mature phalaris plants had a significantly higher root to shoot ratio than the CNG mature plants, the response of phalaris plants to phosphorus may have been limited by the size of the pots and an inability to grow additional roots to support further stem growth.

The findings from this work show that the grass species both respond to phosphorus in a similar manner, when grown separately. Although the grasses were grown in individual pots, without the effects of interspecific competition, the results indicate that CNG is able to respond to soil phosphorus much like introduced grasses, such as phalaris. This finding challenges recommendations made in management guides for the use of introduced grasses such as phalaris as competitive species for limiting invasion of and replacing CNG without the incorporation of other management that place additional stresses on CNG (Bedggood 2000; Bedggood and Moerkerk 2002; Campbell 1995; 1997; Hill 1997). The competitive interactions between such species need to be examined in mixed swards and under grazed conditions.

4.5.3. Plant response to soils of differing pH

The effect of soil pH on phalaris, and the sensitivity of its growth to acid soils, is well documented in comparison to what is known about the effect of soil pH on CNG. Phalaris is known to prefer neutral to slightly basic soils (GRDC *et al.* 1998; Morrow and Brown 1997; Ridley and Coventry 1992; Ridley and Windsor 1992) whereas CNG has been observed to grow in acid soils (Alston and Alston 2003). However, in this study both CNG and phalaris seedlings in the neutral pH soil had significantly larger leaf areas, and tended to have heavier shoots than plants in the acid or alkali soils. Mature plants of both species also benefited from higher pH soils as they had heavier shoots and larger leaf areas, although shoot height was reduced during early growth.

The seedlings of both species responded in a similar manner to changes in soil pH, implying that manipulation of soil pH alone will not favour the growth of a particular species during seedling growth. In the latter phases of growth for these species, phalaris responded better to increasing soil pH than CNG, in terms of shoot weight and shoot height.

4.5.4. Interaction between soils with added phosphorus and differing soil pH

Soil pH differentially affects the availability of nutrients such as phosphorus and nitrogen for plant growth (GRDC *et al.* 1998). Such an interaction between pH and phosphorus became evident in seedling heights from day 19 onwards and for mature plants from day 12 to day 26.

Where no phosphorus was added, the weight of plants and total leaf area, especially for phalaris, in the neutral pH soil was significantly greater than that of the plants in the acid or alkali pH soil.

In comparison, with additional phosphorus, plant heights were the same across all pH treatments, and both CNG and phalaris mature plants had the heaviest shoots and largest leaf areas at pH 9. This implies that the amount of phosphorus added to the soil was to the point of saturation and completely met plant demand, such that phosphorus was non limiting within all pH treatments. This also means that at high phosphorus levels, pH is likely to have less effect on growth.

4.5.5. Damage to plants by nitrogen application

The foliar application technique for the nitrogen treatments caused leaf burn of seedlings and stunted their growth when compared with no nitrogen treatments, whereas this technique was adequate to apply the additional nutrient solution and phosphorus treatment. To avoid plant damage it is advisable to directly apply the nitrogen solution to the soil with the possibility of using more split applications to reduce the nitrogen concentration.

4.5.6. Future work

The response of phalaris seedlings to the addition of phosphorus is potentially a tool that can be used to encourage pasture competition against CNG during seedling development, although the response of CNG to soil phosphorus during reproductive growth was significantly greater than that of phalaris. The timing of phenological development needs to be clearly established to utilise differences in growth stages between CNG and phalaris as a management tool.

In this experiment interspecific competition between these two species was not investigated but this is an area that needs to be examined, especially with the plants growing together using the same nutrient resources. With its greater root to shoot ratio, phalaris may have a competitive advantage and more so in the field.

4.6. Conclusion

Phalaris seedling growth was encouraged more by applying phosphorus to the soil when compared with CNG seedlings, however by maturity, the response of CNG to soil phosphorus was greater than that of phalaris, indicating the use of increased phosphorus with phalaris may not be useful of itself in generating increased plant growth responses against CNG.

The growth responses of the two species were similar in soils of differing pH, so as with phosphorus, changing the soil pH is unlikely to be useful management tool for CNG, at least when in a mixed sward with phalaris.