Plant and Soil
2014, 374:109–119
DOI 10.1007/s11104-013-1870-2

Nitrogen form but not elevated CO_2 alters plant phosphorus acquisition from sparingly soluble phosphorus sources

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Abstract
***Background and aims*:** Maintaining nutrient supply, including phosphorus (P), is critical to ensure the adaptation of cropping systems to future elevated CO_2 (eCO_2) environments. There is much speculation about the role of sparingly soluble sources to supply plants with P so we tested the hypothesis that eCO_2 increases plant's ability to utilise P from sparingly soluble sources via affecting rhizosphere properties.
***Methods*:** Chickpea and wheat were grown for 6 weeks in washed sand supplied with 40 mg P kg^{-1} as either readily soluble $Ca(H_2PO_4)_2$ or sparingly soluble $AlPO_4$ (Al-P), $FePO_4$ or hydroxyapatite (HAP). Half plants were exposed to eCO_2 (700 ppm) while the others to ambient CO_2 (380 ppm).
***Results*:** Elevated CO_2 increased biomass production of both species but did not influence P concentration in plants, rhizosphere pH or Olsen P. Among the sparingly soluble P sources, HAP resulted in the maximum biomass and total P uptake in wheat and chickpea with wheat acquiring more P. Supply of nitrate, as compared to urea, to wheat decreased the uptake of P from HAP but increased it from Al-P.
***Conclusion*:** Elevated CO_2 does not specifically affect plant access to P from sparingly soluble P sources. Urea facilitates P acquisition from HAP whereas nitrate facilitates it from Al-P.

Key words: Climate change, Genotypic variation, P uptake, Rhizospere pH, Root morphology

Introduction
In most soils, inorganic P (Pi) in soil solution ranges from 0.1 to 10 μM and this low concentration limits plant growth (Raghothama 1999; Frossard et al. 2000). Inorganic P in soil can be sorbed to aluminium (Al) and iron (Fe) in acid soils with high concentrations of trivalent Fe and Al, or to calcium (Ca) in alkaline soils where Ca is the major cation. These processes decrease the availability of P to plants (Hinsinger 2001; Turner et al. 2007). Although soluble P fertilizers can be applied to alleviate the P deficiency, most of this applied

P itself would be subsequently adsorbed or precipitated as amorphous or crystalline forms in the soil (Lambers et al. 2006; Richardson et al. 2009).

Many plant species are able to access P from sparingly soluble P sources in P-deficient soils using a variety of mechanisms including a high density of root hairs, release of root exudates and rhizosphere acidification (Riley and Barber 1971; Lambers et al. 2006). However, elevated atmosphere CO_2 (eCO_2) has not been recognized as an abiotic factor that may potentially facilitate P acquisition via affecting root morphology and/or rhizosphere characteristics (Jin et al. 2012). Elevated CO_2 can alter root morphology. For example, eCO_2 increased the number of clusters, length of lateral roots and total root dry weight of P-deficient lupins (*Lupinus albus*), compared with ambient CO_2 (aCO_2) (Watt and Evans 1999; Campbell and Sage 2002), allowing lupins to explore greater soil volumes. Chemical processes in the rhizosphere that mobilize sparingly soluble P may also be altered under eCO_2. Changes in root exudation under eCO_2 may favour desorption of P from Al and Fe oxides via a ligand exchange reaction in rhizosphere (Geelhoed et al. 1999). Furthermore, these changes under eCO_2 may also alter rhizosphere pH, which in turn influences the solubility of sparingly soluble P sources in soil. However, it is unclear whether sparingly soluble P in soils can be mobilized to increase P availability to plants under eCO_2. It is expected that enhanced root proliferation and changes in rhizosphere properties under eCO_2 could enhance P utilization from sparingly soluble P.

Plant species differ in their growth response to and their ability to mobilise P under eCO_2. For example, Stöcklin and Körner (1999) reported that under P deficiency, eCO_2 did not increase the above-ground community biomass when legumes were absent, while biomass increased by 14% when legumes were present. These various responses of plant species to eCO_2 may be due to their different capabilities to absorb P from the soil or differential tolerance to P deficiency (Ghannoum et al. 2006). Rhizosphere acidification by N_2-fixing legumes may enhance P mobilization in soil and subsequent P acquisition in legumes in comparison to non-legumes (Hinsinger et al. 2003; Tang et al. 1997). Nevertheless, the ratio of root to total plant biomass was higher in wheat than in chickpea when P was deficient (Pearse et al. 2007), and the difference between the two species on root biomass varied with P addition (Betencourt et al. 2012), indicating that root traits are also the key factor to expand access to sparingly soluble P. Thus, P-efficient species may benefit more from eCO_2 than P-inefficient ones in P-deficient soils.

Nitrogen (N) form is another significant factor affecting plant P acquisition. Wang et al. (2011) reported that with soluble P application, the NH_4^+-fed cotton, wheat and white lupin plants had greater shoot P uptake compared to NO_3^--fed plants, and that wheat efficiently utilised Al-P when nitrate was applied. Gahoonia et al. (1992) also showed that adding NH_4^+ to ryegrass markedly decreased rhizosphere pH and depleted HCl-extractable P. This raises the question as to how N form influences plant access to P from sparingly P sources, because N-induced change of rhizosphere pH may greatly affect the solubility of P in soil.

This study aimed to investigate how eCO_2 and N form influence plant ability to access P from sparingly soluble P sources and to assess differences in the P-mobilizing capability between chickpea and wheat, two important crops in Australian farming systems. We hypothesized that eCO_2 favoured P mobilization from sparingly P sources via changing root traits and rhizosphere pH, and N form would affect the magnitude of P mobilization through regulating rhizosphere pH. Moreover, we proposed that the two species would differ in their ability to

mobilize sparingly soluble P sources due to differences in root traits and rhizosphere acidification.

Materials and Methods

Experimental design and plant culture

Experiment 1

The experiment consisted of two levels of atmospheric CO_2, four P sources and two plant species in a split-plot design with CO_2 as the main plot, and P and plant species as sub-plot treatments. There were four replications for each treatment. The P treatments included one soluble P (Ps) as $Ca(H_2PO_4)_2$ and three sparingly soluble sources: viz. $AlPO_4$ (Al-P), $FePO_4$ (Fe-P) and hydroxyapatite (HAP), plus a control without P supply (P0). All of these P sources were synthesised compounds with 99.9% of purity (Sigma-Aldrich). Phosphorus was applied at 40 mg P kg^{-1} soil. The two CO_2 levels were aCO_2 (380 ppm) and eCO_2 (700 ppm). Chickpea (*Cicer arietinum* L. cv. Genesis 836) and wheat (*Triticum aestivum* L. cv. Beaufort) were used as they represent major grain legume and cereal crops, respectively, in Australia.

Three point five kg of fine sand (< 2 mm) were loaded into each PVC column (30 cm high × 10 cm diameter) lined with a plastic bag to prevent leaching of solution. The siliceous sand (Maddingley, Victoria, Australia) was washed with tap water before used for this experiment. The chemical properties of the sand were pH 6.2 (1:5 in 0.01 M $CaCl_2$), total C 0.55 μg g^{-1}, total N 0.03 μg g^{-1} and Olsen P 2.5 μg g^{-1}. The sand was mixed with basal nutrients at the following rates (μg formula g^{-1}): $CaCl_2.2H_2O$, 150; K_2SO_4, 140; $MgSO_4.7H_2O$, 20; $MnSO_4.H_2O$, 15; $ZnSO_4.7H_2O$, 9; $CuSO_4.5H_2O$, 2; H_3BO_3, 0.7; $Na_2MoO_4.2H_2O$, 0.2; FeEDTA, 5.5. The different P sources were added to the sand and thoroughly mixed before the sand was added to each PVC column. Nitrogen was supplied in the 1st and the 5th week at the rate of 30 μg N g^{-1} as $(NH_2)_2CO$ (urea) after planting.

Nine uniform germinated seeds of each species were sown at a depth of 2 cm in each column. The seedlings were thinned to 4 plants for chickpea and 5 plants for wheat per column 1 week after sowing. The plants were grown for 6 weeks in CO_2-controlled rooms in a naturally lighted glasshouse (two rooms for aCO_2 and two for eCO_2). Using Guardian Plus of infrared gas monitors (Edinburgh Instruments, Linvingston, UK), atmospheric CO_2 was continually monitored and regulated (via a solenoid) at the required concentration (ambient = 380 or eCO_2 = 700 ppm) with ±2.5% of accuracy. The average irradiance during the experiment was 9.0 MJ m^{-2} d^{-1}. Each room was equipped with air-conditioner to maintain a temperature of 21 ±2 °C. Columns of two replications were randomly allocated in one room and rotated weekly between two rooms of the same CO_2 treatment. Plants were watered every 2 days with Reverse osmosis water to 80% of field capacity by weighing.

Experiment 2

The first experiment showed that both chickpea and wheat were efficient in utilising P from HAP irrespective of CO_2 treatment. The results are inconsistent with the findings of our previous study showing that wheat efficiently utilized P from Al-P (Wang et al. 2010). However, the two studies used different forms of N and different wheat varieties. Wang et al. (2010) used the wheat variety Yitpi and $Ca(NO_3)_2$ as the N source. To confirm the findings of the first experiment, a second column experiment was conducted. The experiment consisted of three P sources, two N forms, two wheat varieties and three replicates. The P treatments included 0 (P0) and 40 μg P g^{-1} in the form of Ps, Al-P, or HAP. Urea or $Ca(NO_3)_2$ was supplied as a rate of 30 μg N g^{-1} at the 1st and the 5th week after planting. Two wheat

varieties, Beaufort and Yitpi were used in this experiment and plants were grown in a controlled-environmental cabinet at 22 °C (day, 12 h) and 18 °C (night) for 6 weeks with a light intensity (over the waveband 400–700 nm) of 210 μmol photons m^{-2} s^{-1}. Basal nutrient addition and watering were the same as Experiment 1.

Plant harvest and measurements

Plastic bags were removed from the PVC columns and roots were gently removed from the bulk sand. The sand adhered to the roots were considered as 'rhizosphere' sand, and collected by shaking root system (Maschner et al. 2004). 'Bulk' sand (containing no obvious roots) was also sampled. All sand samples were mixed thoroughly and air-dried before further analysis.

The pH was determined using a Thermo Orion 720 pH meter in 0.01 M $CaCl_2$ (1:5 = w:v). Plant available P concentration of the sand was measured as described by Olsen and Dean (1965).

Plants were separated into shoots and roots by cutting at sand level. Shoots were rinsed once in 0.1 M HCl and then twice in deionized water. Roots were washed with tap water until free of sand, and then soaked in 0.01 M $CaCl_2$ solution for 5 min to desorb nutrients on root surface. The root length was determined by a root analysis system, WinRHIZO (Mac Rhizo Pro version 2003b, Régent Instruments Inc., Québec, CA). All plant samples were then oven-dried at 70°C for 72 h, weighed and ground. Subsamples were digested with a mixture of nitric and perchloric acid (4:1) (Yuen and Pollard 1954). The concentration of P in the digests was colorimetrically measured using malachite green (Motomizu et al. 1983).

Statistical analysis

GenStat for Windows (Version 12 VSN International software for bioscience) was used to perform statistical analyses. Protected ANOVA tests of LSD were used to assess the differences between treatment means (Steel and Torrie 1980). The data were statistically analyzed by 3-way ANOVA to determine the effects of CO_2/N, P, species/cultivar and their interactions. Standard errors are presented along with means.

Results

Plant growth under eCO_2

Shoot dry weight of chickpea varied among treatments ranging from 180 to 460 mg $plant^{-1}$, while wheat showed a variation from 50 to 260 mg $plant^{-1}$. Elevated CO_2 significantly increased shoot dry weight of chickpea with all P sources and of wheat with Ps (Figure 1A, Table 1). Applying P as either Ps or HAP increased shoot dry weights of both chickpea and wheat compared to the P0 control with the increase being greatest for HAP, followed by Ps (Figure 1A, Table 1). Although overall shoot dry weight was lower for wheat than for chickpea, the wheat plants had a greater proportional response to P applied as Ps and HAP, and this resulted in a significant P × species interaction (Table 1).

Elevated CO_2 also increased ($P < 0.05$) root dry weight of chickpea in all the P treatments, but increased root weight of wheat only under Ps, resulting in a significant species × CO_2 interaction (Figure 1B, Table 1). The root dry weight of chickpea and wheat responded differently to the P sources; the chickpea roots had their lowest biomass in the Ps treatment, whereas wheat supplied with HAP produced a greater root biomass than wheat supplied with other P sources (Figure 1B).

Plant P concentration and uptake under eCO_2

Neither elevated CO_2 nor species affected shoot P concentration in any P treatment. However, the P sources Ps and HAP significantly increased shoot P concentration in both species ($P < 0.001$), while the application of Al-P and Fe-P had no effect on P concentration, compared with the P0 control (Figure 2A, Table 1). Shoot P concentrations were very high in the Ps treatment (exceeding 8 mg g^{-1}) in both species. The wheat plants again had a greater proportional increase to P applied as Ps and HAP above the P0 control, compared to chickpea, and this resulted in the significant P × species interaction (Table 1). The P concentrations in roots followed a similar pattern (Figure 2B). Chickpea had higher P concentrations in the roots compared to wheat ($P<0.05$) (Table 1).

Elevated CO_2 increased the total P uptake (roots plus shoots) by around 15% across all P treatments for both species (Figure 2C and Table 1). Total P uptake was greater for Ps and HAP than the P0 control, the Al-P and Fe-P sources. Chickpea had higher P uptake than wheat. Adding Ps or HAP produced greater increases in P uptake in wheat than chickpea, resulting in a significant P × species interaction ($P < 0.001$) (Table 1).

Root traits under eCO_2

Elevated CO_2 significantly increased the root length of chickpea regardless of P source. In wheat, eCO_2 increased root length only when P was applied (Table 1, Figure 3A). Plant species differed significantly in their response to the P sources (Table 1). In chickpea, the increase of root length occurred only with HAP compared with the P0 control, while in wheat, all P treatments resulted in the increase of root length with the maximum increase of 93% occuring with HAP (Figure 3A). In addition, eCO_2 did not affect specific root length across any P treatments (overall average 50 m g^{-1} root).

Elevated CO_2 did not alter the P uptake per unit of root length regardless of P sources or species (Figure 3B, Table 1). Nor was there any main effect of species. However, when Ps or HAP was applied, the P uptake per unit of root length increased significantly compared with the P0 control. The increase with Ps was greater in chickpea, whereas the increase with HAP was greater in wheat, resulting in the significant P × species interaction (Table 1).

pH and P availability in the rhizosphere under eCO_2

Rhizosphere pH was not affected by either CO_2 treatment or species (Figure 4A, Table 1). However, the rhizosphere pH was significantly higher when Ps or HAP was applied compared to the other P treatments (Figure 4A, Table 1).

Olsen P in the rhizosphere was approximately 6 times higher with Ps than the P0 control, but was only slightly higher when Al-P or HAP was applied compared to Fe-P and the P0 control (Figure 4B, Table 1)

3.5. Plant growth in response to N and P sources

The wheat cv. Yitpi produced greater shoot dry weight than cv. Beaufort. There was a significant N source × P treatment interaction ($P < 0.001$) (Table 2), with urea increasing shoot dry weight when either Ps or HAP was applied, while nitrate increased shoot dry weight only when Al-P was applied (Table 3). The P × cultivar interaction was also significant with the Yitpi plants having a greater response to the application of Ps, Al-P and HAP than Beaufort plants (Tables 2, 3).

There were significant 2-way interactions among N form, P source and cultivar on the root dry weight of wheat (Table 2). In addition, there was a significant N × cultivar interaction (Tables 2, 3), where Yitpi had a greater root biomass response to nitrate than Beaufort.

P concentration and uptake response to N and P sources

The application of Ps and HAP increased shoot P concentration compared with the P0 control, and urea N resulted in higher shoot P concentration than nitrate N (Tables 2, 4). There was a significant N source × P form interaction ($P < 0.001$), where urea increased shoot P concentration with Ps and HAP form, whereas nitrate resulted in higher shoot P concentration with the Al-P form.

N source and P form affected root P concentration differently compared to shoot P concentration (Table 4). When Ps was applied, nitrate produced higher root P concentrations than when urea N was applied, the opposite to what occured in the shoots. However, urea still increased root P concetration to a greater extent than nitrate with HAP but to a lesser extent than urea with Al-P, resulting in a significant N × P interaction ($P < 0.001$) (Table 2).

There was a significant interaction between N and P on total P uptake (Table 2). Urea resulted in greater total P uptake in the supply of Ps and HAP whereas nitrate led to higher total P uptake in the Al-P treatment. Total P uptake was higher in Yitpi than in Beaufort ($P < 0.001$) (Table 4). Yitpi also had a greater increase in total P uptake when P was supplied as Ps and HAP.

Root length response to N and P sources

The effect of the P source on root length depended on the N form, leading to a significant N × P interaction (Table 2). Compared with urea N, nitrate N produced a 94% increase in root length with Al-P, whereas N form did not affect root length when either Ps or HAP was applied (Table 3).

Both the form of N and P type markedly affected specific root length of wheat plants with urea increasing the specific root length more than nitrate, and Al-P and HAP increasing specific root length compared to the Po control (Table 3). A significant N × P interaction occurred (Table 2), with urea resulting in higher specific root lengths than nitrate with Ps, Al-P and HAP, while the reverse occurred with the P0.

Urea resulted in greater P uptake of wheat per unit root length than nitrate, and the Ps and HAP forms resulted in more P uptake per unit root length than the P0 and Al-P treatments (Table 4). Again there was a significant N × P interaction ($P < 0.001$) (Table 2). Compared with nitrate, adding N as urea almost doubled P uptake per unit root length for Ps while adding HAP resulted in a 4- to 5-fold increase.

Discussion

Although eCO_2 did increase plant growth, especially that of chickpea, it did not increase the ability of either species to access P from sparingly soluble P sources. It was evident that neither the plant P concentration nor the P uptake per unit of root length was affected by eCO_2 in either species across the different P sources assessed (Figures 2, 3). These results are consistent with the finding that eCO_2 does not increase P concentrations in chickpea, field pea or wheat when grown in a P-deficient Vertisol and Calcarosol (Jin et al. 2012; 2013). Further evidence for the lack of any eCO_2 effect was that eCO_2 affected neither Olsen-P (plant available P) concentration nor rhizosphere pH (Table 1, Figure 4), indicating that eCO_2

was unlikely to enhance mobilization of P from insoluble forms by changing rhizosphere pH. Thus, the overall effect of eCO_2 on the utilization of P from sparingly soluble P sources was limited.

Although eCO_2 increased total P uptake when HAP was supplied to chickpea, it did not promote P mobilization from HAP. This enhanced P uptake reflected the increased biomass under eCO_2. Elevated CO_2 resulted in a 17% increase of shoot dry weight of chickpea under HAP and a similar increase in P uptake, but did not change P concentration in shoots or roots. Further, a significant linear relationship between P uptake and biomass ($P < 0.001$) was found in this study (data not shown). Similar results were observed by Jin et al. (2012) who found that increases in P uptake under eCO_2 resulted from increased biomass production, rather than from changes in the specific ability of the roots to take up P from the soil.

Unlike eCO_2, P source strongly influenced plant growth and P uptake. An interesting phenomenon was that the growth of both chickpea and wheat with Ps, the water-soluble P form, was less than with the HAP form. This could be explained by P toxicity occurring in the Ps treatment. The P concentration in the shoots of both species with Ps was around 8 mg g^{-1} (Figure 2), which exceeds the toxic critical level (7 mg g^{-1}) for these growth stages (Reuter and Robinson 1997). Thus, the growth response to Ps is likely to have been constrained by P toxicity in the plant tissue. The major reason for this toxicity can be attributed to the low P-buffering capacity of the sand (Bolland et al. 1994).

In this study, both species mobilised more P from HAP than from Al-P and Fe-P, while the plants had mobilised little P from Al-P as indicated by minimal P uptake. This finding contrasts with that from a previous study which showed that wheat could acquire P from Al-P, rather than HAP, when wheat plants were also grown in sand (Wang et al. 2010). Pearse et al. (2006; 2007) also stated that wheat mobilized P from Al-P more effectively than from Fe-P and HAP. This discrepancy can be explained by the N form that was used in these different studies. Our first experiment used urea to supply N while the experiments carried out by Pearse et al. (2006; 2007) and Wang et al. (2010) used nitrate as the sole N source.

The effect of N form on P acquisition was confirmed by the second experiment, where urea increased P acquisition from HAP whereas nitrate increased P uptake from Al-P (Table 4). The basis for this effect can be explained by the effect of the N form on the release of ions to the rhizosphere. Urea can be hydrolysed to form NH_4^+ in the sand solution and the subsequent uptake of NH_4^+ by the crop roots, results in the release of H^+ from root system (Zoysa et al. 1998) to maintain charge balance across the root membranes. In this present study, the rhizosphere pH was lower with supply of urea (pH = 6.3) than nitrate (pH = 6.7). Indeed, rhizosphere pH with urea supply might well have been lower in soil as the sand had an extremely low adsorption capacity for protons, and a follow-up study showed that watering of the columns led to leaching and diffusion of protons from the rhizosphere into bulk sand. Acidification promotes the dissolution of HAP (Bertrand et al. 1999; Hinsinger and Gilkes 1996; Zoysa et al. 1998). In contrast, the extrusion of OH^- and HCO_3^- anions counter-balances the uptake of NO_3^-, when nitrate is supplied (Wang et al. 2010). We propose that this is likely to facilitate Al-P dissolution. This effect results when OH^- reacts with some Al from Al-P to form $Al(OH)_3$. This reaction will occur because the solubility of $Al(OH)_3$ ($Ksp = 4.6 \times 10^{-33}$) is considerably lower than the solubility of Al-P ($Ksp = 6.3 \times 10^{-19}$), and $Al(OH)_3$ is the predominant Al-hydrolysis species in soil solution when pH is above 6.3 (Ma et al. 2003). Thus the rhizosphere alkalization that occurs with nitrate supply favours the formation of $Al(OH)_3$ which in turn drives the dissolution of P from Al-P, thereby improving

the effectiveness of Al-P as a P source. This proposed mechanism can also explain the results of Wang et al. (2010) and Pearse et al. (2006, 2007) who found that Al-P was an effective P source for wheat when N was supplied in the nitrate form.

The two N forms also had different effects on the growth of wheat roots in the second experiment where there was a highly significant N form × P source interaction for root growth and root function. The key comparisons here are between the effective combinations of simultaneously applying nitrate-N with Al-P, or urea-N with HAP. The wheat plants responded to the nitrate-N / Al-P combination by producing a large root mass , long roots and somewhat thicker roots (smaller specific root lengths) (Table 3). Although there were more roots with nitrate-N and Al-P compared to the urea-N / HAP combination, the P uptake per unit root length was low (Table 4). In contrast, wheat plants supplied with urea-N and HAP produced a lower root mass and a similar root length (Table 3), but an increased P uptake per unit of root length– compared to the nitrate-N/Al-P combination. Thus the contrasting responses were that the root proliferated with nitrate-N and Al-P but the roots took up less P per unit root length, whereas with urea-N and HAP, there was overall a smaller root system but with greater P uptake per unit root length. Many previous studies investigated the N-regulated root developmental processes, which contribute to the differences in root morphology. It has been reported that NO_3^- stimulates lateral root elongation (Forde 2002), while NH_4^+ accelerates cell division and subsequent root branching (Bloom et al. 2003). Although it is not clear just what the significance of these responses might be, they do highlight how root growth and P uptake capacity are affected by the chemical environment in the soil surrounding the roots.

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There were a number of genetic differences in the ability to acquire P from the different P sources. The first was between wheat and chickpea, where urea-fed wheat responded more to HAP relative to the P0 control, than did chickpea. This resulted in the significant species × P source interactions for plant growth and P accumulation. Although chickpea had a heavier root system with longer roots (Figures 1B, 3A), wheat was able to take up more P from HAP than chickpea (Figure 3B). Furthermore, wheat had a higher shoot P concentration with HAP than chickpea (Figure 2A) and a greater P uptake per unit root length (Figure 3B). These findings highlight the effectiveness of wheat in accessing P from HAP compared to chickpea, considering that the plants are smaller, with smaller seeds and lower seed P content, than chickpea. The reason for the superior ability of wheat to mobilize P from HAP remains unknown. In a previous study, Pearse et al. (2006) showed wheat had less carboxylate concentrations in its rhizosphere compared to chickpea irrespective of P sources.

There were further genetic differences between the two wheat cultivars used in the second experiment where they differed in the ability to acquire P from the P sources. Compared with Beaufort, Yitpi had greater P uptake when combinations of either Al-P plus nitrate or HAP plus urea was supplied (Table 4). This result may reflect the higher root biomass and the longer roots of Yitpi than Beaufort (Table 3). These genotypic differences in root proliferation may enhance P acquisition from sparingly soluble (inorganic) P sources.

The rhizosphere pH of wheat and chickpea that were supplied with HAP in the first experiment were greater than these for the Po control treatments (Figure 4A). One might have expected the pH to decrease in line with the uptake of NH^+-N derived from the urea, and the resulting release of H^+-ions by the plant roots (Bertrand et al. 1999; Hinsinger and Gilkes 1996). The unexpected result can be explained by the consumption of H^+ in dissolving HAP. Loganathan et al. (1995) stated that 2 moles of H^+ were consumed for every mole of P

dissolved from rock phosphate, of which HAP is a major constituent (Pearse et al. 2006). The HAP used in this study was a pure form of hydroxyapatite, without free carbonate that might react with H^+ ions. This further supports that H^+ ions were largely consumed in the dissolution of HAP in this study, leading to increased rhizosphere pH.

Conclusions

Irrespective of plant species, eCO_2 did not affect plant P concentration, P uptake per unit root length or P solubility in the rhizosphere when P was supplied in different forms, indicating that eCO_2 does not enhance the mobilization of P from sparingly soluble (inorganic) P sources by either chickpea or wheat. Thus the inevitable future increase in global atmosphere CO_2 will not result in any significant alleviation of P deficiency by crops via increased access to non-labile soil P. On the other hand, N form influenced plant's ability to access P from these P sources in sand culture. Compared with nitrate, urea substantially increased P concentration and total P uptake from HAP, whereas nitrate enhanced P mobilisation from Al-P. Such an effect of N form could be attributed to plant N metabolism altering root development and rhizosphere pH.

Acknowledgement

This research was supported by an Australian Research Council *Linkage Project* (LP100200757) in partnership with the Department of the Environment and Primary Industries (Victoria).

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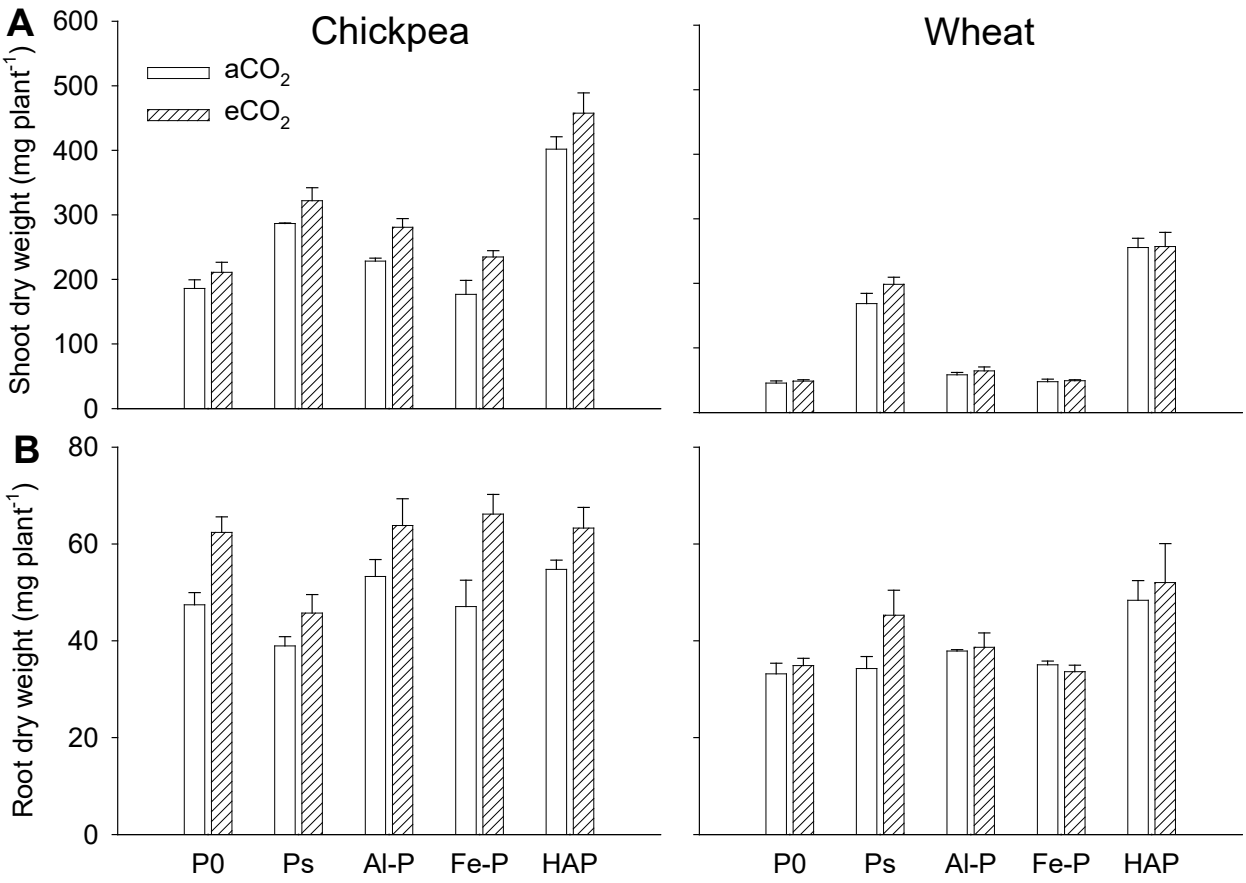

Figure 1 (Expt. 1). Dry weights of shoots (A) and roots (B) of chickpea and wheat supplied with either 0 (P0) or 40 mg P kg^{-1} soil as $(H_2PO_4)_2$ (Ps), $AlPO_4$ (Al-P), $FePO_4$ (Fe-P) and hydroxyapatite (HAP) under ambient (aCO_2, 380 ppm) or elevated CO_2 (eCO_2, 700 ppm) for 6 weeks. All plants were supplied with urea. Error bars represent the standard error (n=4).

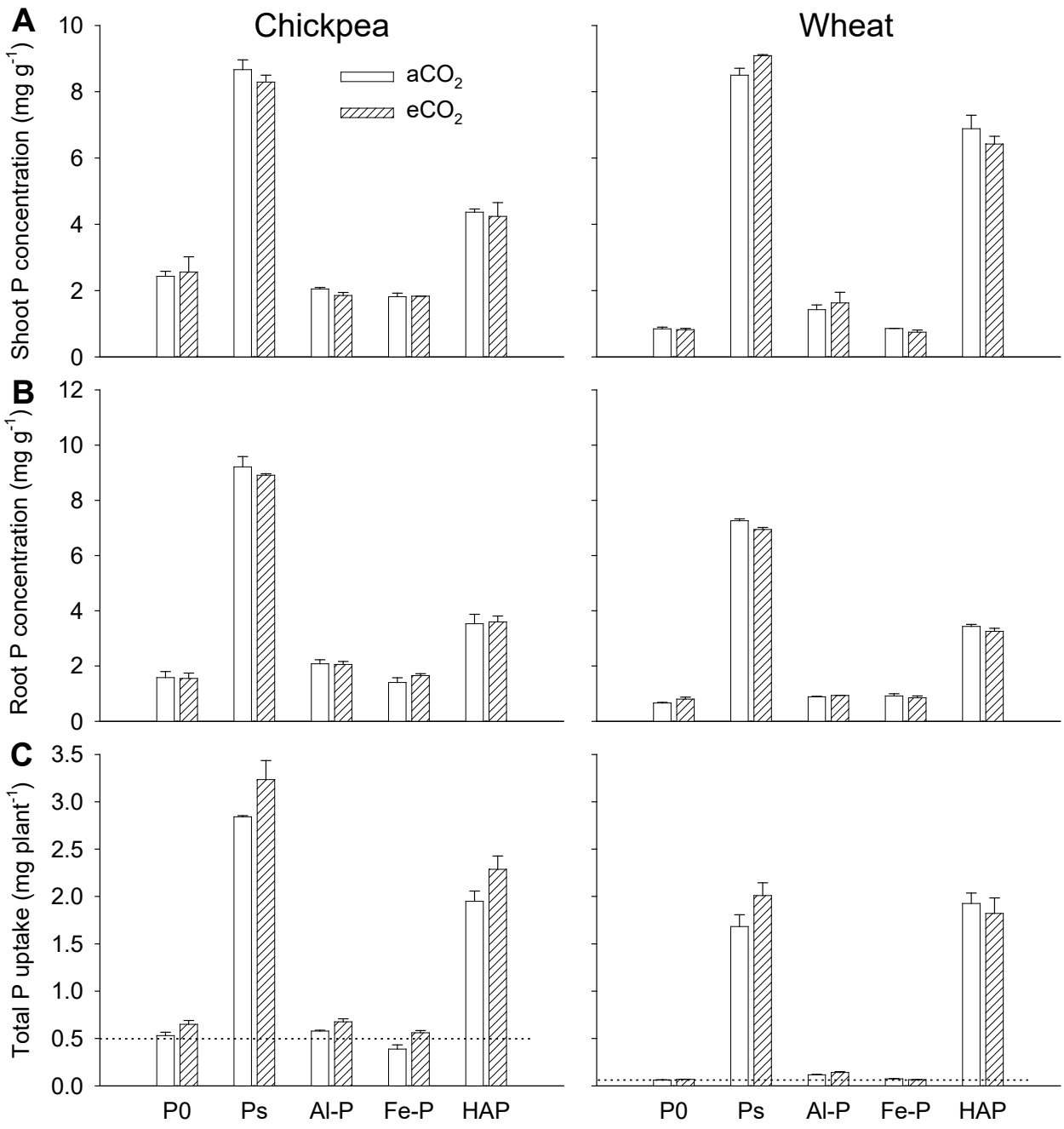

Figure 2 (Expt. 1). Phosphorus concentration in shoots (A) and roots (B), and total P uptake (C) of chickpea and wheat supplied with either 0 (P0) or 40 mg P kg^{-1} soil as $Ca(H_2PO_4)_2$ (Ps), $AlPO_4$ (Al-P), $FePO_4$ (Fe-P) and hydroxyapatite (HAP) under ambient (aCO_2, 380 ppm) or elevated CO_2 (eCO_2, 700 ppm) for 6 weeks. All plants were supplied with urea. The dotted lines represent seed P content. Error bars represent the standard error (n=4).

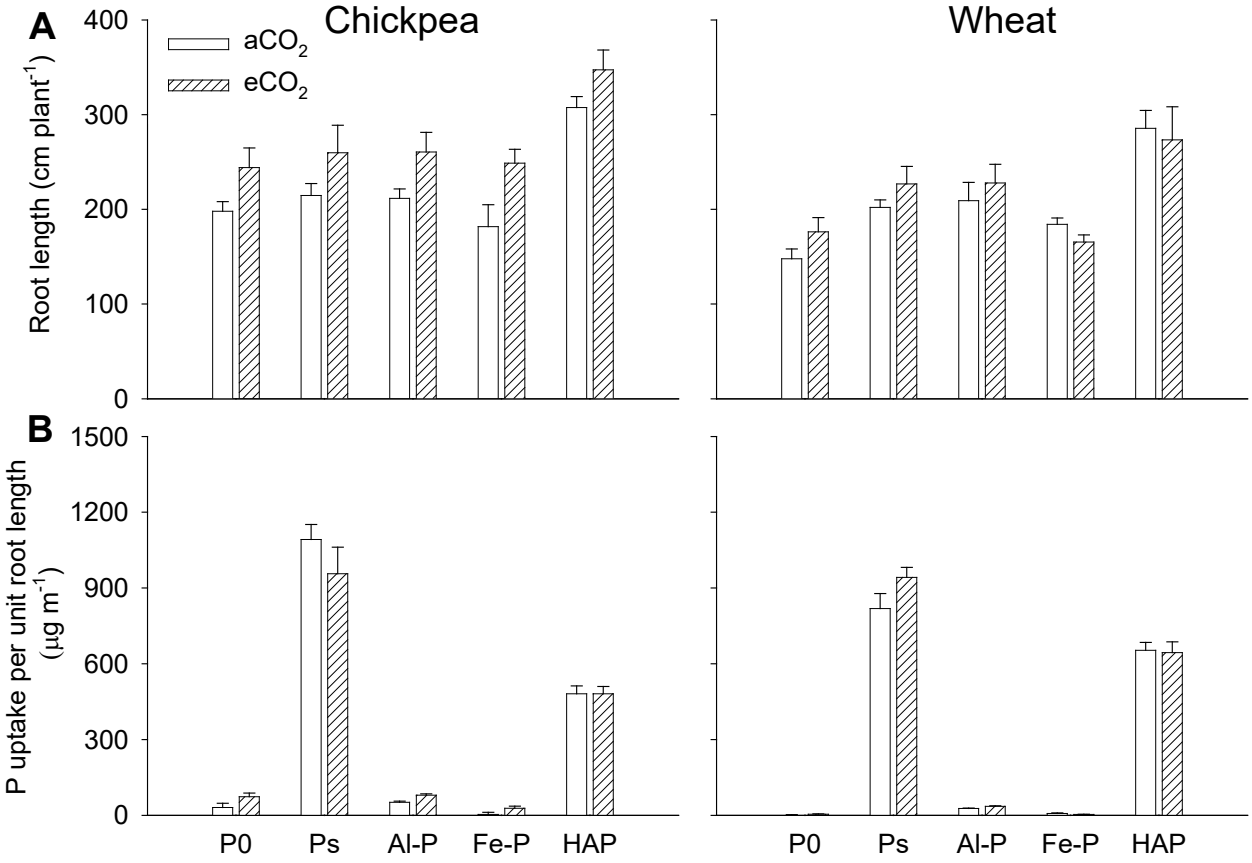

Figure 3 (Expt. 1). Root length (A) and P uptake per unit root length (B) of chickpea and wheat supplied with either 0 (P0) or 40 mg P kg^{-1} soil as $Ca(H_2PO_4)_2$ (Ps), $AlPO_4$ (Al-P), $FePO_4$ (Fe-P) and hydroxyapatite (HAP) under ambient (aCO_2, 380 ppm) or elevated CO_2 (eCO_2, 700 ppm) for 6 weeks. All plants were supplied with urea. The seed P has been removed in the calculation of P uptake per unit root length. Error bars represent the standard error (n=4).

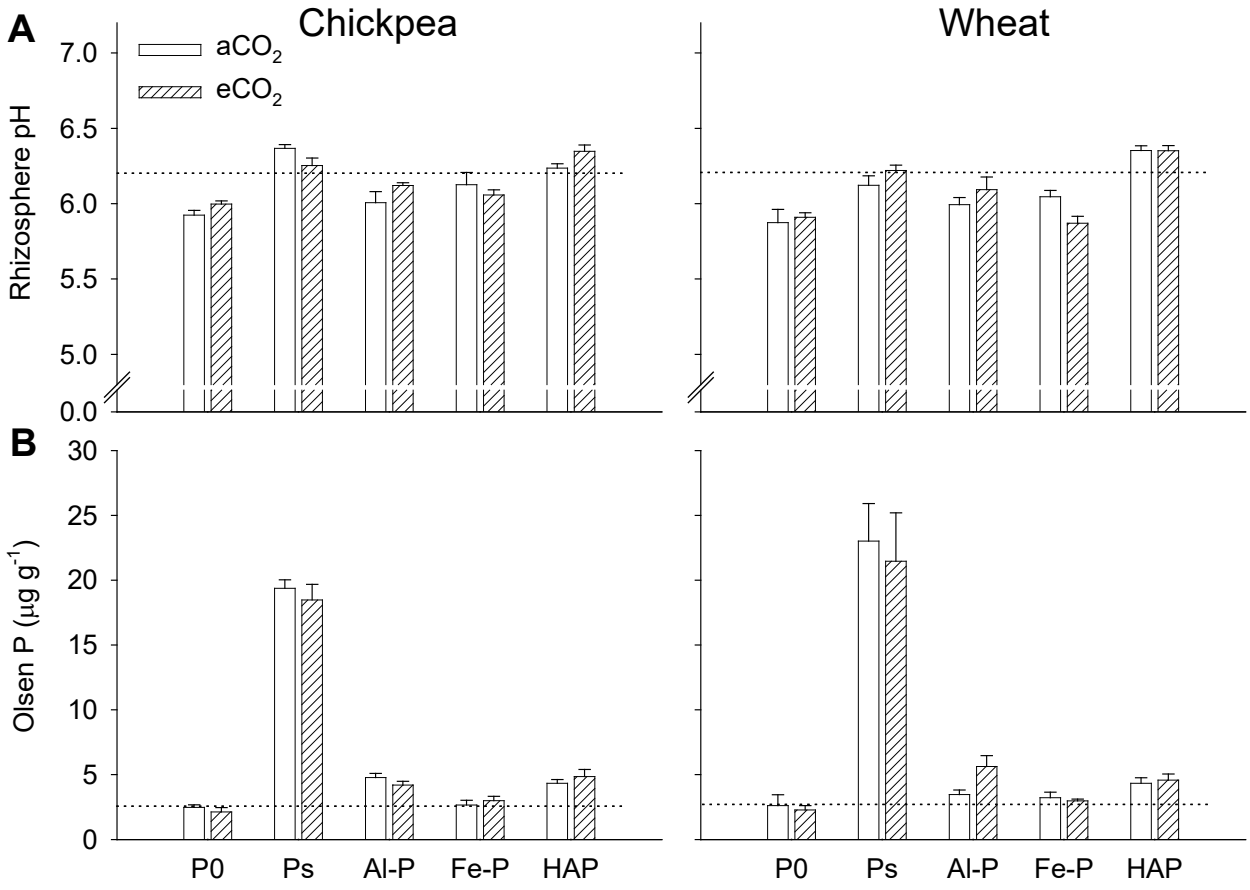

Figure 4 (Expt. 1). The pH (A) and plant available (Olsen) P (B) in the rhizosphere of chickpea and wheat supplied with either 0 (P0) or 40 mg P kg^{-1} soil as $Ca(H_2PO_4)_2$ (Ps), $AlPO_4$ (Al-P), $FePO_4$ (Fe-P) and hydroxyapatite (HAP) under ambient (aCO_2, 380 ppm) or elevated CO_2 (eCO_2, 700 ppm) for 6 weeks. All plants were supplied with urea. The dotted lines represent the initial pH and Olsen P of the sand medium. Error bars represent the standard error (n=4).

Table 1 (Expt. 1). Significant levels of main effects and interactions of CO_2, P sources (P) and species on shoot and root dry weights (DW), shoot and root P concentration, total P uptake, root length, P uptake per unit root length, rhizosphere pH and Olsen P.

Factors	Shoot DW	Root DW	Shoot P concentration	Root P concentration	P uptake	Root length	P uptake per unit root length	Rhizosphere pH	Olsen P
CO_2	*	*	n.s.	n.s.	*	*	n.s.	n.s.	n.s.
P	***	**	***	***	***	*	***	***	***
Species	***	***	n.s.	***	***	***	***	n.s.	n.s.
CO_2×P	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
CO_2×Species	n.s.	***	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.
P×Species	***	***	***	*	***	*	***	n.s.	n.s.
CO_2×P×Species	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

*, **, *** and n.s. indicate $P < 0.05$, $P < 0.01$, $P < 0.001$ and $P > 0.05$, respectively.

Table 2 (Expt. 2). Significant levels of main effects and interactions of P and N forms, and wheat cultivar on dry weights (DW) of shoot and roots, shoot and root P concentration, total P uptake, root length, specific root length (root length per unit root biomass) and P uptake per unit root length.

Factors	Shoot DW	Root DW	Shoot P concentration	Root P concentration	P uptake	Root length	Specific root length	P uptake per unit root length
N	***	***	***	*	***	***	***	***
P	***	***	***	***	***	***	**	***
Cultivar	***	***	n.s.	*	***	***	**	n.s.
N×P	***	***	***	***	***	***	***	***
N×Cultivar	n.s.	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
P×Cultivar	***	***	n.s.	*	***	**	*	n.s.
N×P×Cultivar	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.

*, **, *** and n.s. indicate $P < 0.05$, $P < 0.01$, $P < 0.001$ and $P > 0.05$, respectively.

Table 3 (Expt. 2). Effect of N form on dry weight of shoots and roots, root length, specific root length (root length per unit root biomass) and P uptake per unit root length of wheat (cvs. Bearufort and Yitpi) grown for 6 weeks without P (P0) or with P supplied as $Ca(H_2PO_4)_2$ (Ps), $AlPO_4$ (Al-P), hydroxyapatite (HAP) at a rate of 40 mg P kg^{-1} soil. The N forms are urea and nitrate with a rate of 30 mg N kg^{-1} supplied at the 1st and the 5th week after sowing, respectively.

Cultivars	P sources	Shoot DW (mg $plant^{-1}$)		Root DW (mg $plant^{-1}$)		Root length (cm $plant^{-1}$)		Specific root length (m g^{-1})	
		Urea	Nitrate	Urea	Nitrate	Urea	Nitrate	Urea	Nitrate
Beaufort	P0	62	73	33	49	241	456	73	94
	Ps	314	216	44	51	533	450	121	89
	Al-P	91	154	33	74	394	632	121	86
	HAP	349	136	42	64	545	645	130	101
Yitpi	P0	61	80	30	44	281	553	94	126
	Ps	420	382	65	101	762	787	118	78
	Al-P	117	266	42	117	598	1292	143	110
	HAP	439	198	60	99	855	887	143	89
	LSD (p=0.05)	54		18		204		22	

Table 4 (Expt. 2). Effect of N form on concentration of P in shoots and roots, and total P uptake of wheat (cv. Bearufort and Yitpi) grown for 6 weeks without P (P0) or with P supplied as $Ca(H_2PO_4)_2$ (Ps), $AlPO_4$ (Al-P), hydroxyapatite (HAP) at a rate of 40 mg P kg^{-1} soil. The N forms are urea and nitrate with a rate of 30 mg N kg^{-1} supplied at the 1st and the 5th week after sowing, respectively. Seed P contents were 0.07 mg $plant^{-1}$ for Beaufort and 0.10 mg $plant^{-1}$ for Yitpi.

Cultivars	P sources	Shoot P conc. (mg g^{-1})		Root P conc. (mg g^{-1})		Total P (mg $plant^{-1}$)		P uptake per unit root length (μg m^{-1})	
		Urea	Nitrate	Urea	Nitrate	Urea	Nitrate	Urea	Nitrate
Beaufort	P0	1.39	1.18	0.84	0.86	0.11	0.13	47	28
	Ps	12.3	6.07	4.41	6.48	4.04	1.64	758	364
	Al-P	1.66	3.32	1.32	1.66	0.19	0.63	49	100
	HAP	5.67	1.99	2.91	1.22	2.10	0.35	385	54
Yitpi	P0	1.33	1.27	0.93	0.83	0.11	0.14	39	25
	Ps	12.5	5.69	4.17	5.63	5.52	2.74	725	349
	Al-P	2.36	2.90	1.38	1.73	0.33	0.97	56	75
	HAP	5.54	1.87	2.43	1.37	2.58	0.51	302	57
	LSD (p=0.05)	0.69		0.40		0.53		63	