
Effects of soil moisture content and arbuscular mycorrhizal fungi on phosphorus fractions in soil

Chudaeng, D. and Teamkao, P.*

Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Thailand.

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Abstract The results of the investigation showed that soil moisture significantly affected P-water, P-loosely, P-Fe and P-apatite. P-loosely and P-apatite contents were higher than the control, while P-water and P-Fe contents were high at 75% of soil water holding capacity (WHC) after 90 days of experiment. Arbuscular mycorrhizal fungi (*Glomus* sp. and *Acaulospora* sp.) inoculation significantly differed in P-Al, P-Ca and P-apatite contents. AMF inoculation reduced amounts of P-Al, P-Ca and P-apatite in soil. Interaction between soil moisture and AMF significantly differed in P-loosely and P-apatite with. P-Fe, P-Ca and P-apatite increased while P-water, P-loosely and P-Al decreased. It showed that drought stress reduced P-water content, while AMF addition reduced unavailable phosphorus forms of P-Al, P-Ca and P-apatite in soil.

Keywords: Soil moisture, Arbuscular mycorrhizal, Phosphorus fractions

Introduction

Droughts are major natural hazards that can destroy agricultural areas, deplete water resources and adversely impact on the environment (Sternberg, 2011). Droughts were abundant during the year 2000s, with long-term water shortages in the Western United States, Southeast Australia and Northeast China. Recently, short-term water shortage appeared but severe events occurred in Russia and the Central United States. The cause of drought may emanate from climate change, with the potential to worsen with high temperatures and precipitation changes (Sheffield and Wood, 2008; Dai, 2010). Many studies have attributed to the severity and drought duration to global warming (Briffa *et al.*, 2009; Cai *et al.*, 2009). Droughts intensity has escalated over wider areas since the 1970s, particularly in the tropics and subtropics. Increased drying linked with higher temperatures and decreased precipitation has contributed to change in drought (IPCC, 2007). Drought increase in the last few decades in both regions (Briffa *et al.*, 2009; Wang *et al.*, 2010) and global (Dai *et al.*, 2004) that commensurate with the increase in global temperatures. Under drought conditions, the amount of

* **Corresponding Author:** Teamkao, P.; **Email:** patrarat.te@kmitl.ac.th

water in plants decreases leading to limited growth and causes physiological changes in metabolic processes. Drought adversely affects nutrient availability in the soil (Munns, 1993). Ratios of $\text{Na}^+/\text{Ca}^{2+}$, Na^+/K^+ , $\text{Ca}^{2+}/\text{Mg}^{2+}$ and $\text{Cl}^-/\text{NO}_3^-$ were shown to be higher under saline soil conditions with decreased plant growth due to ionic toxicity such as Na and Cl and imbalances (Grattan and Grieve, 1999). To mitigate these effects, soil quality improvements must be developed, verified and implemented in agricultural practices (Pavol, 2015). Presently, arbuscular mycorrhizal fungi (AMF) are being used in agriculture. Fungi that live in roots positively impact many aspects of plants, such as increase nutrient availability and resistance to inappropriate environments.

As an essential but often limited nutrient, phosphorus (P) plays a central role in food production. The efficient P management is a key to improve food security (Tilman *et al.*, 2002; Syers *et al.*, 2008). Phosphorus limitation in agroecosystems is usually overcome by applying P fertilizers to the soil surface. However, excessive soil application of P fertilizer can cause ecological and economic problems such as reducing rock phosphate in nature, P absorption capacity and leaching or runoff of P fertilizer from agricultural land. Plants uptake P from the soil solution as ionic orthophosphate (H_2PO_4^- or HPO_4^{2-}) via roots or mycorrhizal hyphae (Pierzynski and McDowell, 2005). Soil solution contains low concentrations of available P (Achat *et al.*, 2016), but can be replenished with P from the soil solid phase to provide additional uptake by plants (Pierzynski and McDowell, 2005). Therefore, P exchange kinetics or soil solution is replenished by P from the soil solid phase, an important implication for P requirement of living organisms (Fardeau *et al.*, 1991; Menezes *et al.*, 2016).

Drought stress reduces P content in plants by 9.18% and shows stronger effects in short term (less than 90 days) than in the long term. Moreover, drought stress has a negative effect on P in plants (He and Dijkstra, 2014). The dry conditions in Mediterranean shrubland caused by forest fire has reduced soil P immobilization (Hinojosa *et al.*, 2012). Drought reduce 15% soil moisture and reduce P in above-ground biomass of *Quercus ilex* by 33% wherein the soluble inorganic P to soluble organic P is by 50% (Sardans and Penuelas, 2007). In Evergreen Mediterranean forest, 20% soil moisture reduced P in above-ground of plant at 40%, inorganic labile P in soil at 48% (Sardans and Penuelas, 2004).

Arbuscular mycorrhizal fungi (AMF) are symbiotic and mostly occurring in at least 80% of vascular plant families. AMF facilitate plant uptake of mineral nutrients such as phosphorus and nitrogen by increasing the absorbing surface area and mobilizing sparsely available nutrients. In turn, plant hosts supply AMF with a carbon source that is essential for fungal growth (Wanxiao *et al.*, 2017). Meanwhile, AMF help plants to survive under inappropriate environments by increasing their drought tolerance and resistance to plant diseases which improve plant growth

(Schenck, 1981). However, benefits of AMF on plants depend on soil properties, plant and fungal species. (Smith and Read, 2000; Tawara *et al.*, 2001). Non-agricultural soil will have higher amounts of AMF spores compared to agricultural soil (Gravito and Miller, 1998). Moreover, low or very high phosphorus content reduces soil AMF accession to the roots (Koide, 1991; Clark, 1997). AMF colonize plant roots and release phosphatase to the soil. This increases phosphorus availability to plants by transforming the phosphorus from unavailable form to available form (Tarafdar and Marschner, 1994). Available phosphorus in the soil relates to fixation and dissolution of inorganic phosphorus which may be related to soil moisture. The aims of research finding were investigated the effects of different soil moisture contents, and AMF addition to soil on the change of seven inorganic P forms as available P (P-water and P-loosely) and unavailable P (P-Al, P-Fe, P-reductant, P-Ca and P-apatite).

Materials and Methods

Experimental design

The experiment was designed as 4×2 factorial in completely randomized design (CRD) with three replications. The first factor was soil moisture content as 25% (highly drought), 50% (moderate drought) and 75% (non-drought) defined as soil water holding capacity (WHC) (Vicente *et al.*, 2012) and the control where water was not added to the soil after being air-dried (1.37% moisture content). The second factor was inoculation of arbuscular mycorrhizal fungi eg. *Glomus* sp. and *Acaulospora* sp. with 25 active spores/g and non-inoculation with AMF. It was conducted in plastic pots four inches in diameter using 800 g/pot of Ultisol soil and 1 g of AMF for inoculation treatment. The soil was air dried and passed through 2 mm sieve before application. The non sterilized soil was measured as pH 5.98, 0.27 mS/cm EC, 1.07% organic matter, 52.97 mg/kg available phosphorus, 46.03 mg/kg extractable potassium, 1,481.67 mg/kg extractable Ca and 145 mg/kg extractable Mg). The experiment was done for 90 days in a greenhouse. Soil moisture was kept by weighing and addition of distilled water as necessary. P-fraction analysis was determined by random samplings at 0, 30, 60 and 90 days after incubation.

Soil P-fraction analysis

Phosphorus in soil was extracted consecutively for seven P fractions according to plant availability for P-water, P-loosely, P-Al, P-Fe, P-reductant, P-Ca and P-apatite following the method of Kuo (1996). P-water was done using 1.0 g (< 2 mm) soil and dissolved in 25 ml distilled water in a 50 ml centrifuge tube and shaken for 30 min. Then, the filtrate or clear

part was filtered through Whatman filter paper No.42 (extract A). P-loosely was done using 25 ml of 1 M NH_4Cl added to the residue and the suspension was shaken for 30 min, and centrifuged at 2,500 rpm for 10 min and filtered through Whatman filter paper No.1 (extract B). P-Al was done using 25 ml of 0.5 M NH_4F (pH 8.2) added to the residue and the suspension was shaken for 1 h and centrifuged at 2,500 rpm for 10 min and filtered through filter paper No.1 (extract C). The soil was washed twice with 12.5 ml saturated NaCl and centrifuge at 2,500 rpm washings were combined with extract C. P-Fe was done using 25 ml of 0.1 M NaOH added to the residue and the suspension was shaken for 17 h, centrifuged at 2,500 rpm for 10 min and through filter paper No.1 (extract D). Subsequently, the soil was washed twice with 12.5 ml saturated NaCl and centrifuge at 2,500 rpm, washings were combined with extract D. P-reductant was done using 20 ml of 0.3 M $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$ and 2.5 ml of 1 M NaHCO_3 added to the residue and the suspension was heated in a water bath at 85 °C. The sample was centrifuged at 2,500 rpm for 10 min and filtered through filter paper No.1 (extract E). The soil was washed twice with 12.5 ml of saturated NaCl and centrifuged at 2,500 rpm, washings were combined with extract E. P-Ca was done using 25 ml of 0.25 M H_2SO_4 added to the residue and the suspension was shaken for 1 h, centrifuged at 2,500 rpm for 10 min and filtered through filter paper No.1, the soil was washed twice with 12.5 ml of saturated NaCl and centrifuge at 2,500 rpm, washings were combined with extract F. P-apatite was done using 25 ml of 1 M HCl added to the residue and the suspension was shaken for 1 h, centrifuged at 2,500 rpm for 10 min and filtered through filter paper No.1, the soil was washed twice with 12.5 ml of saturated NaCl and centrifuge at 2,500 rpm, washings were combined with extract G.

An aliquot was contained 1 to 10 ml of each extract A, B, C, D, E, F and G, separately transferred into 25 ml volumetric flasks. Distilled water and three drops of p-nitrophenol indicator was added to the volumetric flasks containing extracts D, F and G, and pH was adjusted by 2 M HCl or 2 M NaOH until the indicator color changed. 7.5 ml of 0.8 M H_3BO_3 was added to volumetric flask containing extract C. Phosphorus concentration in the various solutions were determined by following the ascorbic acid method (Murphy and Riley, 1962).

Statistical analysis

Statistical analysis was performed using analysis of variance (ANOVA). The least significance difference (LSD) test was used to establish whether the differences in the treatments were significant at 95% confidence level.

Results

P-fractions on the day of the experiment

Analysis of P-fractions on the day of experiment found that moisture content of the soil significantly affected P-Al and P-Fe at $P < 0.05$. The P-Al and P-Fe contents were highest for 50% WHC at 23.34 and 6.63 mg/kg, respectively. The lowest concentration of P-Al and P-Fe were recorded in the control of 20.15 and 5.61 mg/kg, respectively. AMF addition was affected P-water and reduced P-water content from 4.77 mg/kg to 3.68 mg/kg ($P < 0.05$). Interaction between soil moisture and AMF addition did not result in different phosphorus content in any P-fractions (Table 1.).

Table 1. P-fraction contents in soil at the beginning experiment

Factor	P-fraction (mg/kg)						
	P-water	P-loosely	P-Al	P-Fe	P-Ca	P-apatite	
% WHC	control	3.45	8.75	20.15 C	5.61 B	1.26	-
	25%	3.95	10.26	20.76 BC	6.23 A	1.01	-
	50%	5.01	9.91	23.34 A	6.63 A	1.06	-
	75%	4.49	10.24	22.41 AB	6.24 A	1.10	-
AMF	+ ^{1/}	3.68 b ^{2/}	10.04	21.72	6.15	1.12	-
	-	4.77 a	9.54	21.61	6.20	1.09	-
% WHC	ns ^{3/}	ns	**	**	ns	-	
AMF	*	ns	ns	ns	ns	-	
% WHC × AMF	ns	ns	ns	ns	ns	-	
% CV	28.57	18.55	6.30	6.06	16.13	-	

^{1/}+: AMF inoculation; -: no AMF inoculation

^{2/} different capital letters indicate statistical difference among treatments

^{3/} ns: non-significant; *: significant at 95% probability level; **: significant at 99% probability level

P-fractions on 30th day of the experiment

Analysis of P-fractions at 30 days found that soil moisture content affected P-water, P-loosely, P-Fe, P-Ca and P-apatite with significant differences at $P < 0.05$. The highest were P-loosely and P-Fe in control at 8.25 and 7.75 mg/kg, respectively. The highest P-Ca, P-apatite, and P-water contents were found at 25%, 50% and 75% WHC with 4.66 mg/kg, 6.51 mg/kg and 3.73 mg/kg, respectively. Addition of AMF did not affect P-fractions but interaction between soil moisture and AMF addition impacted on P-loosely, P-Fe and P-Ca (Table 2).

Table 2. P-fraction contents in soil on day 30th of the experiment

Factor	P-fraction (mg/kg)						
	P-water	P-loosely	P-Al	P-Fe	P-Ca	P-apatite	
% WHC	control	2.35 B ^{2/}	8.25 A	11.07	7.75 A	4.46 A	2.52 B
	25%	2.22 B	5.44 B	10.81	7.37 A	4.66 A	0.73 C
	50%	3.53 A	4.96 B	10.56	5.82 B	2.08 B	6.51 A
	75%	3.73 A	6.30 B	11.69	5.74 B	2.82 B	0.30 C
AMF	+ ^{1/}	3.30	6.57	11.11	6.76	3.27	2.78
	-	2.61	5.90	10.96	6.57	3.74	2.25
% WHC	* ^{3/}	**	ns	**	**	**	
AMF	ns	ns	ns	ns	ns	ns	
% WHC × AMF	ns	**	ns	*	**	ns	
% CV	30.41	19.26	8.46	7.70	25.22	31.44	

^{1/} +: AMF inoculation; -: no AMF inoculation

^{2/} different capital letters indicate statistical difference among treatments

^{3/} ns: non-significant; *: significant at 95% probability level; **: significant at 99% probability level

P-fractions on 60th day of the experiment

P-fractions at 60 days found that moisture content in the soil significantly affected P-water, P-loosely, P-Al, P-Fe and P-apatite at $P < 0.05$. The highest P-water, P-loosely, P-Al and P-Fe contents were found at 75% WHC with 5.91, 8.57, 17.72 and 6.26 mg/kg, respectively. AMF addition affected to P-water and P-loosely with significant difference at $P < 0.05$. The AMF inoculation increased P-water from 2.87 mg/kg to 4.28 mg/kg but decreased P-loosely from 5.60 to 4.86 mg/kg. Interaction between soil moisture and AMF addition affected P-apatite content with significant differences ($P < 0.05$) (Table 3).

P-fractions on day 90th of the experiment

P-fractions at 90 days found that moisture content in the soil significantly affected P-water, P-loosely, P-Fe and P-apatite at $P < 0.05$. The highest P-loosely and P-apatite were found at control with 7.22 mg/kg and 2.92 mg/kg, respectively. However, the highest P-water and P-Fe at 75% WHC were 3.37 mg/kg and 7.65 mg/kg, respectively. AMF addition affected P-Al, P-Ca and P-apatite. Non-inoculation gave higher P-Al, P-Ca and P-apatite at 16.82 mg/kg, 4.09 mg/kg and 2.01 mg/kg, respectively. Interaction between soil moisture and AMF addition affected P-loosely and P-apatite (Table 4).

Table 3. P-fraction contents in soil on day 60 of the experiment

Factor	P-fraction (mg/kg)						
	P-water	P-loosely	P-Al	P-Fe	P-Ca	P-apatite	
% WHC	control	0.87 C ^{2/}	3.13 C	13.68 C	5.37 C	2.88	0.37 B
	25%	3.22 B	4.27 B	15.22 B	5.71 B	3.25	0.80 A
	50%	4.29 B	4.95 B	16.08 B	6.08 A	4.03	0.40 B
	75%	5.91 A	8.57 A	17.72 A	6.26 A	3.14	0.79 A
AMF	+ ^{1/}	4.28 a	4.86 b	15.24	5.75	3.35	0.50
	-	2.87 b	5.60 a	16.11	5.97	3.30	0.68
% WHC	** ^{3/}	**	**	**	ns	*	
AMF	*	*	ns	ns	ns	ns	
% WHC × AMF	ns	ns	ns	ns	ns	**	
% CV	36.56	16.11	6.45	4.67	22.72	33.79	

^{1/}+: AMF inoculation; -: no AMF inoculation^{2/} different capital letters indicate statistical difference among treatments^{3/} ns: non-significant; *: significant at 95% probability level; **: significant at 99% probability level**Table 4.** P-fraction contents in soil on day 90 of the experiment

Factor	P-fraction (mg/kg)						
	P-water	P-loosely	P-Al	P-Fe	P-Ca	P-apatite	
% WHC	control	1.87 B ^{2/}	7.22 A	16.12	5.49 B	2.32	2.92 A
	25%	1.84 B	5.50 B	17.14	6.29 B	3.97	0.82 B
	50%	3.37 A	5.43 B	15.27	7.21 A	3.79	1.18 B
	75%	3.37 A	4.97 B	15.78	7.65 A	3.11	0.84 B
AMF	+ ^{1/}	2.89	5.39	15.33 b	6.57	2.51 b	0.87 b
	-	2.33	6.17	16.82 a	6.75	4.09 a	2.01 a
% WHC	* ^{3/}	**	ns	**	ns	**	
AMF	ns	ns	*	ns	*	**	
% WHC × AMF	ns	*	ns	ns	ns	**	
% CV	42.15	16.22	8.15	10.92	41.33	25.28	

^{1/}+: AMF inoculation; -: no AMF inoculation^{2/} different capital letters indicate statistical difference among treatments^{3/} ns: non-significant; *: significant at 95% probability level; **: significant at 99% probability level

Change in P-fractions

Change in P-fractions showed that P-water was reduced in soil in every treatment (Fig. 1). P-loosely content was reduced in all soil moisture content but increased after 60 days at low soil moisture (25% WHC) and

control. P-Al in soil, as the main P form, dropped at 30 days in all treatments. However, P-Al increased under 25% WHC and control but increased at 60 days and reduced at 90 days under 50% WHC and 75% WHC. P-Fe in soil had no changed, some treatments were constant, but some treatments were fluctuating. P-Ca changed might related to AMF. P-Ca increased in AMF inoculation, but decreased in non-inoculation treatments. P-apatite was increased in all soil moisture contents.

Discussion

P-fractions were separated into labile P as P-water and P-loosely, moderately labile P as P-Al, P-Fe and P-Ca, and non-labile P as P-apatite. Change of phosphorus during the 90 days of incubation under various drought stresses showed reduction of easily usable P-form as P-water in soil. However, usable phosphorus in soil as P-loosely decreased in all soil moisture contents. Higher P-Al and P-Fe contents were found at 50% WHC and 75% WHC than at control and 25% WHC. These two P forms occur from the reaction of phosphate with Al and Fe under acidic condition in soil with available Al and Fe ions. Aluminum is more involved in phosphorus sorption in soils than in sediments (National Research Council, 1993). P-Ca content in soils was not statistically different. Although Ca content in soil was 1,480 mg/kg, but the pH was 5.98 which did not suit for P-Ca formation. The three P-fractions, P-Al, P-Fe and P-Ca were moderately labile. P-reductant as the reduced form of phosphorus was not found in this study. P-reductant is found only under reduction conditions in soil that lacks oxygen or is waterlogged like paddy fields. As the study period increased, a trend of higher P-apatite was found in lower soil moisture content. Apatite is a form of insoluble P that is rarely available for plants. Mingzhu and Feike (2014) reported that drought stress decreased available P at 9.18%. The availability of soil P to plants depends on the replenishment of labile P from other P fractions. Inorganic-P fractions in soils change under a wide range of management conditions (Nwoke *et al.*, 2004).

The effect of drought was shown clearly on P-water but not on P-loosely. Jarosch *et al.* (2018) reported that using water for 10 consecutive extractions could be used to predict P availability for plants more effectively than ammonium lactate, commonly used to determine extractable P in Sweden (Jarosch *et al.*, 2018). Measurement of P via water extraction might be more related to available P status for plants. Phosphorus is relatively enriched in finer soil fractions and perhaps correlate with the clay content of soil. For soil with sandy loam texture, availability of P is at a low level and possibly too low to observe for any change (National Research Council, 1993). AMF addition reduced unavailable phosphorus forms of P-Al, P-Ca and P-apatite in soil. Decrease of P-Al, P-Ca and P-apatite was significant on day 90 of the experiment. However, incubation had no host plant for

AMF to colonize. Activity of AMF in soil during non-host plant needs further investigation.

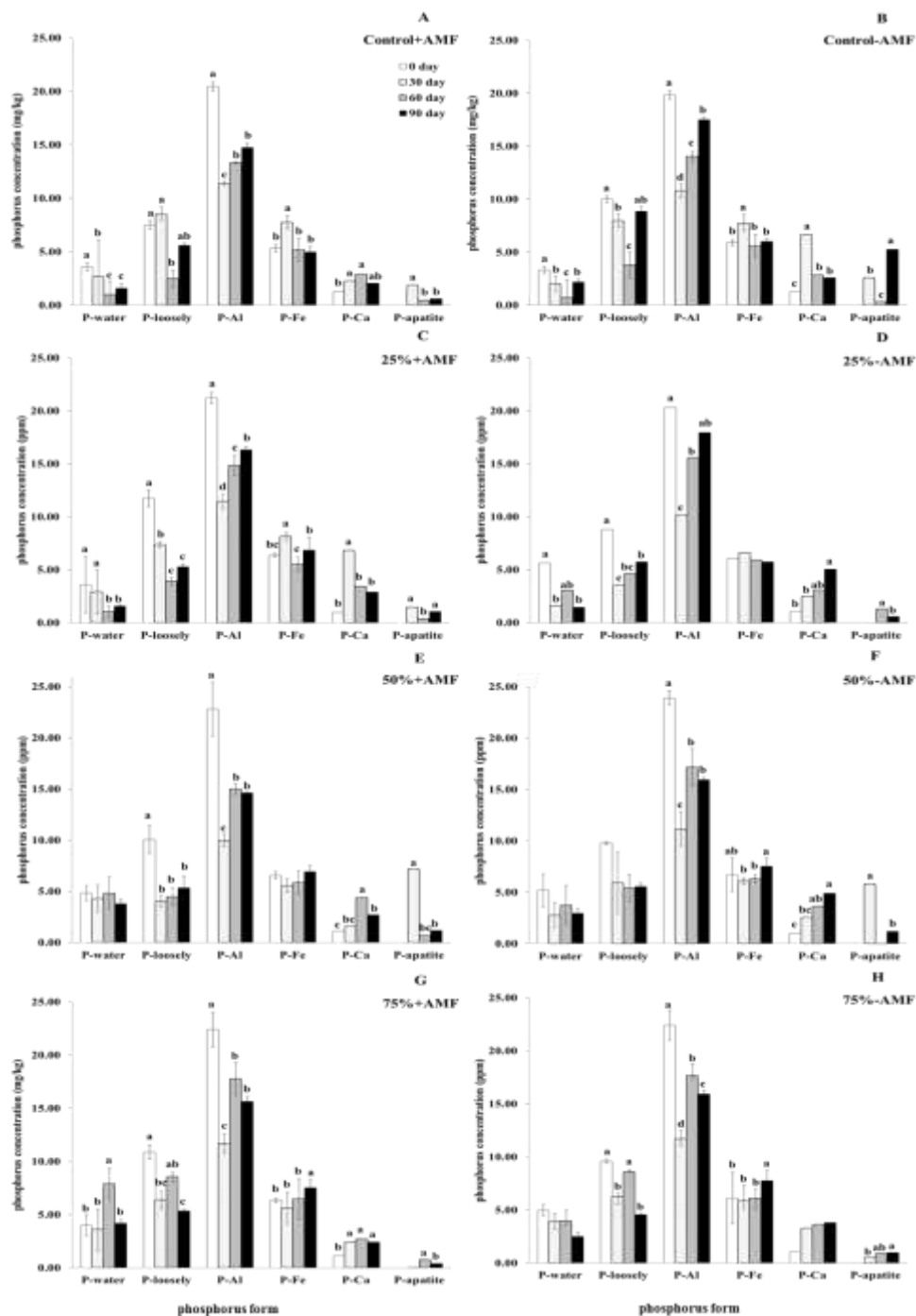


Figure 1. Change in P-fraction during the study period

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