Effects of air-drying and freezing on phosphorus fractions in soils with different organic matter contents

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ABSTRACT

Little is known about the effects of air-drying and freezing on the transformation of phosphorus (P) fractions in soils. It is important that the way in which soils respond to such perturbations is better understood as there are implications for both P availability and loss to surface waters from soils. In this study, the effects of air-drying and freezing were investigated using two soils, one being a forest soil (FS) high in organic matter and the other being a sterile soil (SS) low in organic matter. Soil P was fractionated using a modified Hedley fractionation method to examine the changes of phosphorus fractions induced by air-drying and freezing. Generally, there were no significant differences of total phosphorus among the three treatments (*CV*% < 10%). Compared with field moist soils, freezing the soil evoked few changes on phosphorus fractions except that the resin-P increased in FS soil. On the contrary, air-drying significantly changed the distribution of phosphors fractions for both soils: increased the labile-P (especially resin-P) and organic-P ($NaHCO_3$-Po, NaOH-Po and Con.HCl-Po) at the expense of NaOH-Pi and occlude-P (Dil.HCl-P and Con.HCl-Pi). Resin-P significantly increased by 31% for SS soil and by 121% for FS soil upon air-drying. The effect of air-drying seemed to be more pronounced in the FS soil with high organic matter content. These results indicated that drying seem to drive the P transformation form occlude-P to labile-P and organic-P and accelerated the weathering of stable P pool. This potentially could be significant for soil P supply to plants and P losses from soils to surface waters under changing patterns of rainfall and temperature as predicted by some climate change scenarios.

Keywords: P transformation; Hedley fractionation; air-drying; climate change; forest soil; sterile soil

In many parts of the world climate change means that soils are being exposed to more extreme conditions than those to which they are adapted, e.g. more intense drying or freezing. However little is known about the effects of either of these perturbations on the transformation of phosphorus (P) fractions

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in soils. It is important that the way in which soils respond to such perturbations is better understood as there are implications for both P availability and loss to surface waters from soils. Air-drying is commonly used for long periods storage of P before extraction and analysis, but this was found to increase water and $NaHCO_3$-extractable P in soil compared with field moist soil (Turner et al. 2001, Nguyen and Marschner 2005, Peltovuori and Soinne 2005, Styles and Coxon 2006, Turner et al. 2007, Soinne et al. 2010). Although the mechanisms responsible for this effect are not clear, changes in P fractions and sorption may be important for soil P availability and the quality of water.

Up to now, the disruption of organic matter and the lysis of microbial cells are the two known mechanisms involved in phosphorus release (Laura 1976, Sparling et al. 1985, Qiu and McComb 1995, Turner and Haygarth 2001, Nguyen and Marschner 2005, Zornoza et al. 2009). Air-drying was reported to enhance mineralization of was explained to originate from solubilized organic matter and the disruption of aggregates in soils (Bartlett 1980). Laura et al. (1976) reported that drying may result in chemical breakdown of organic matter thereby increasing P availability. On the other hand, following the air-drying of 15 different New Zealand soils, Sparling et al. (1985) suggested that 76% of bicarbonate extractable reactive P may originate from the microbial biomass-bound phosphorus. Turner and Haygarth (2001) found a positive relationship between the quantities of microbial biomass P in the original soil and the amount of total soluble P they contained after being subjected to drying-rewetting cycles. They confirmed that the increases in water soluble P released from the microbial biomass (Turner and Haygarth 2001, 2003). Furthermore, the flush of mineralized organic matter seemed to be mostly microbial in origin in as much as aggregate distribution was unaffected by air-drying and rewetting cycles (Mikha et al. 2005).

Increases in phosphorus solubility within dried soils following their rewetting are widely reported (Chepkwony et al. 2001). Most studies focused on labile P pools or the P solubility in the water and $NaHCO_3$ extractions (Sardans and Peñuelas 2004). For example, increases in water-extractable organic P of between 185 and 1900% were reported following the air drying of fresh soils (Turner and Haygarth 2001). In a following study, Turner found that $NaHCO_3$-extractable inorganic P concentrations decreased by between 2 and 60% (mean decrease = 21%), while $NaHCO_3$-extractable organic P concentrations increased by between 48 and 156% (mean increase = 95%) following air-drying of fresh soils (Turner et al. 2003). However, there are studies that did not demonstrate the transformations in readily exchangeable P fractions, which would be consistent with the P release in air-drying fresh soils.

Information on the P fractions and transformation upon air-drying is critically needed to get ful understanding of P cycles and long-term changes in P pool especially under global climate change. In this study, a modified Hedley fraction was used to examine the changes of phosphorus fractions induced by air-drying and freezing treatment. The study was performed with different organic matter content soils to test the hypothesis that drying does not only affect the labile P but may also alter the stability of more stable forms of soil P.

MATERIAL AND METHODS

Soil sample. Soils for forest site (FS) and sterile site (SS) were collected to 0–10 cm depth on the east side of the Yantai City (121°26'E, 37°28'N), Shandong Province, China. This region has warm temperate continental monsoon climate with distinctive seasons and rainy summer. The annual average temperature is 12.1°C, the frost-free period lasts 196 days, annual average rainfall is 552 mm, evaporation is 1962 mm, and the drought index is up to 3.56. The major species grown in the forest areas included *Pinus thunbergii Parlatore* and *Firmiana simplex.* The soils are loam to silty loam and high in organic matter. The major group of soils in this region is Calcaric Fluvisols. The other site is unmanaged grassland soil and the soil is poor in available nutrients and low in organic matter (Table 1). After sampling, the soils were stored at 0~4°C, sieved through a 2-mm sieve, homogenized and sub-sampled randomly for three treatments: (i) field moist stored at 0~4°C, for

Table 1. Properties of experimental soil

Soil	SS	FS
Sampling moisture (%)	6.15	15.95
Clay content (%)	3.28	12.52
Organic carbon (%)	0.27	5.31
pH (1:2.5)	6.51	5.49
Total-P (mg/kg)	103	412

SS – sterile site; FS – forest site

Table 2. Sequential extraction procedure, showing extraction reagents and their associated phosphorus (P) fractions (Cross et al. 1995)

Fractions of P extracted	Significance
Resin-P	labile Pi adsorbed on surfaces crystalline compounds
$NaCO_3$-Pi	labile Pi adsorbed on surfaces crystalline compounds
$NaCO_3$-Po	labile Po is easily mineralized and contributes to plant available P
NaOH-Pi	less labile Pi associated with exteriors of amorphous A1 and Fe phosphates
NaOH-Po, Con.HCl-Po	stable Po that is involved with long term transformation of P in soils
Dil.HCl	largely calcium bound
Con.HCl-Pi	less labile Pi associated with interiors of amorphous A1 and Fe phosphates
Redual-P	occluded Pi covered with sesquioxides and other Po

7 days at sampling moisture, (ii) air-drying stored for 7 days at 30°C and (iii) freezing stored for 7 days at –80°C.

Soil analysis. Soil pH was determined with a glass electrode in 1:2.5 soil:water ratio. Organic carbon was determined by combustion on a LECO CNS-2000 auto-analyzer (Elmentar, Halle, Germany). Grain size distributions in the soil samples were determined using a Mastersizer2000 Laser Grain size (Manufactured by Malvern Instruments Ltd., Malvern, UK; measuring range: 0.02–2000 μm). Phosphorus in the extracts and digests was determined by Tu-1810 Spectrophotometer (Manufactured by PERSEE, Beijing, China), using ascorbic acid molybdenum blue method (Murphy et al. 1962). The precision of the methods was approximately 5–10% at the 95% confidence level (Table 1).

A modified sequential Hedley fractionation was used to extracted phosphorus in soils (Hedley et al. 1982, Tiessen and Moir 1993). The fractionation was done in four replicates for each treatment. Generally, soils were extracted step by step using an anion exchange membrane, 0.5 mol $NaHCO_3$, 0.1 mol NaOH, 1 mol HCl and concentrated HCl shaking for 16 h each addition. Finally, residual-P was measured after H_2SO_4/H_2O_2 digestion at 360°C. The $NaHCO_3$, NaOH and concentrated HCl extracts were divided into two aliquots in order to measure the total P and inorganic P. Organic P was calculated as a difference between total and inorganic P (Table 2). Biomass P (Pm) was determined by the fumigation extraction method after Kouno et al. (1995). One-way ANOVA was carried out using the SPSS computer package (SPSS Inc. 1999, Chicago, USA) for all sets of data, and significant differences between means were determined through *LSD* test. Differences were considered statistically significant when $P < 0.05$.

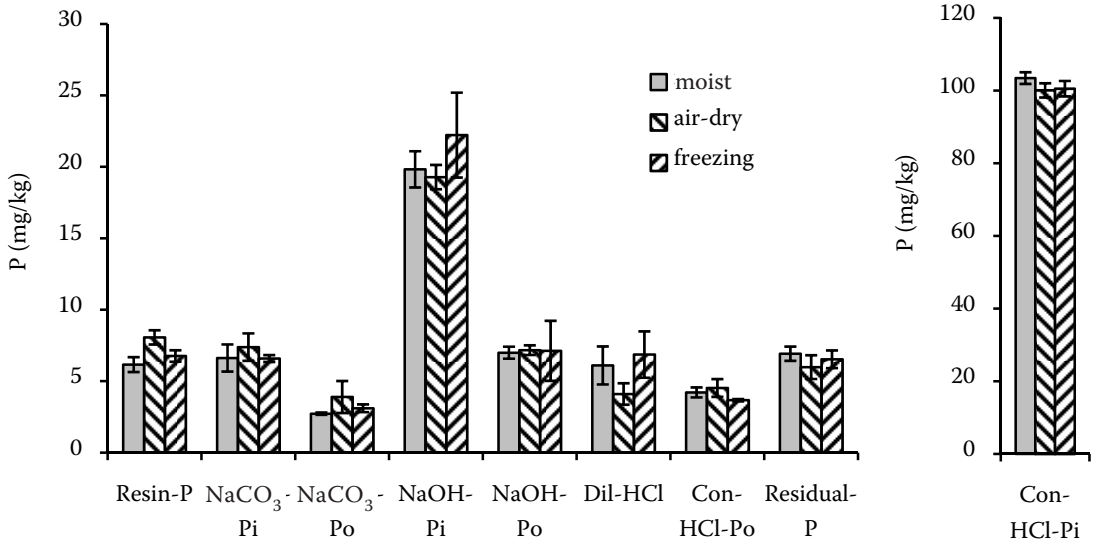

Figure 1. Content of biomass-P, total-P and organic carbon in moist, air-dry and freezing soils for sterile (SS) and forest (FS) sites. Data in the figure indicate means of four replicates (± SD). Different letters on error bars indicate significant difference at $P < 0.05$

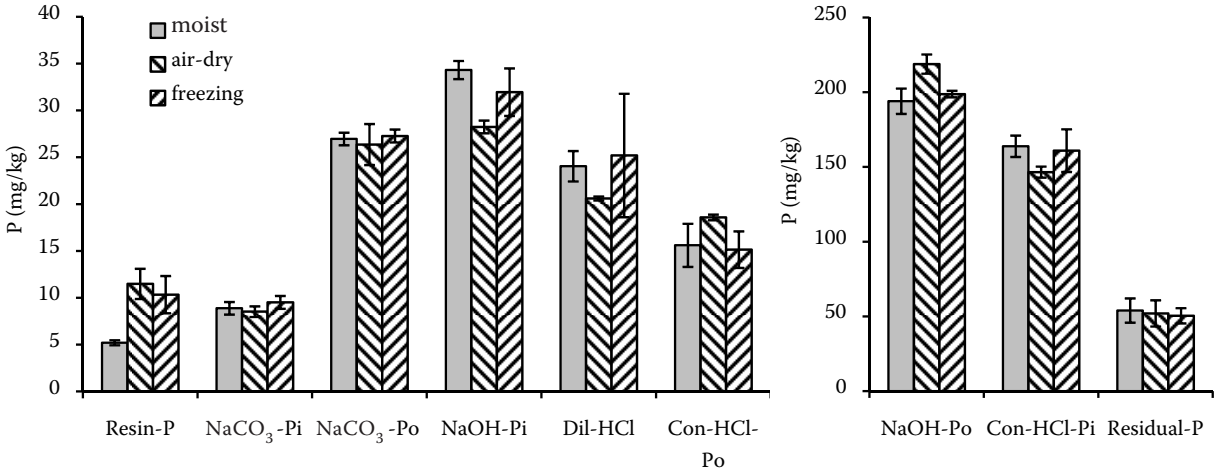

Figure 2. Comparison of phosphorus fractions (mg/kg dry weight) in moist, air-dry and freezing soils for sterile (SS) and forest (FS) sites. Data in the figure indicate means of four replicates (± SD). Different letters on error bars indicate significant difference at $P < 0.05$

RESULTS

There were no significant differences of total phosphorus among the moist, air-drying and freezing sample pretreatments. The variability of the total phosphorus was 5% for SS and 7.6% for FS soil under the storage methods and the data are thus defined as less variable. Compared with moist soils, freezing have little effect on the total organic carbon content (OC%). Conversely, OC% was significantly higher in air-drying soils than that in moist soils, although the increase seems to be more noticeable in SS soils. Biomass-P significantly reduced for both soils whatever the moist soil was air-drying or freezing (Figure 1).

The amount and distribution of the various phosphorus fractions following three treatments (moist, air-drying and freezing) for SS and FS soils are shown in Figure 2 and Table 3, respectively. Generally, freezing the field moist soils had only small effects on the phosphorus fractions (Figure 2, Table 3). On the contrary, air-drying the soils significantly changed the distribution of phosphorus fractions especially the labile-P and organic-P (Figures 1–2). Resin-P content increased significantly when the field moist soils were air-dried at 30°C although the changes were quantitatively and proportionally greater for FS soils than for SS soils. In SS soils, resin-P concentration increased from 6.16 ± 0.52 mg/kg for moist soils to 8.06 ±

Table 3. Comparisons of changes in the relative proportion of phosphorus fractions in sterile soil (SS) and forest soil (FS)

P fractions (n = 4)	SS			Change		FS	Change		
	moist	air-dry	freezing	air-dry (%)	freezing (%)	moist	air-dry	freezing	air-dry (%)	freezing (%)
Resin-P	6.2 (0.5)	8.1 (0.5)	6.8 (0.4)	30.8**	9.7	5.2 (0.3)	11.5 (1.6)	10.3 (2.0)	120.5**	98.5**
$NaCO_3$-Pi	6.6 (1.0)	7.4 (1.0)	6.6 (0.2)	11.5	−0.6	8.9 (0.7)	8.5 (0.6)	9.5 (0.7)	−4.0	7.1
$NaCO_3$-Po	2.7 (0.1)	3.9 (1.1)	3.1 (0.3)	43.0**	14.0	27.0 (0.7)	26.4 (2.2)	27.3 (0.7)	−2.2	1.2
NaOH-Pi	19.8 (1.3)	19.3 (0.9)	22.2 (3.0)	−2.7	12.1	34.3 (1.0)	28.3 (0.7)	32.0 (2.5)	−17.7*	−6.9
NaOH-Po	7.0 (0.4)	7.2 (0.3)	7.1 (2.1)	2.6	1.9	194 (8.5)	218.9 (6.4)	198.8 (2.2)	12.8	2.5
Dil-HCl	6.1 (1.3)	4.1 (0.8)	6.9 (1.6)	−32.8**	12.5	24.1 (1.6)	20.6 (0.2)	25.2 (0.6)	−14.3	4.8
Con.HCl-Po	4.2 (0.4)	4.5 (0.6)	3.7 (0.1)	7.4	−12.8	15.6 (2.3)	18.6 (0.3)	15.2 (2.0)	19.1*	−2.9
Con.HCl-Pi	105.1 (1.6)	100.1 (2.0)	100.5 (2.1)	−4.8	−4.4	163.9 (7.1)	146.57 (3.7)	191.0 (14.3)	−10.6	16.5*
Residual-P	6.9 (0.5)	56.0 (0.8)	6.5 (0.6)	−13.6	−5.6	53.9 (8.1)	52.0 (8.8)	50.4 (5.1)	−3.5	−6.5

*$P < 0.05$;**$P < 0.01$; difference between the air-drying (freezing) and the field moist soil; bracket number indicates standard deviation

0.5 mg/kg for air-dried soils. In the FS soil, the effect of air-drying was even larger. The resin-P concentration results in the air-drying treatment were approximately doubled that in field moist soil. These values increased from 5.12 ± 0.27 mg/kg for moist soils to 11.49 ± 1.61 mg/kg for air-dried FS soils. The $NaHCO_3$–Pi and $NaHCO_3$–Po contents show little variation under air-dry treatment, except for $NaHCO_3$–Po in SS soils where the values increased from 2.72 ± 0.08 mg/kg to 3.89 ± 1.12 mg/kg (Figure 2, Table 3).

Compared to resin-P, organic-P was less sensitive to air-drying treatment. In the SS soil, air-drying increased the content of NaOH-Po from 2.6 to 12.8% and of Con.HCl-Po from 7.4 to 19%. Unlike the labile-P and organic-P, the NaOH–Pi and occlude-P (Dil.HCl-P and Con.HCl-Pi) have the tendency of decreasing concentration following air-dried the both moist soils. Air-drying markedly reduced the Con.HCl-Pi content from 105.11 ± 1.61 mg/kg to 100.06 ± 1.91 mg/kg for SS soils and from 163.86 ± 7.14 mg/kg to 146.57 ± 3.71 mg/kg for FS soils. At the same time, Dil. HCl-P also decreased by 10% and 20% for SS and FS soils, respectively. There were no air-drying induced changes in the residual-P for both soils.

DISCUSSION

Air-drying changed the chemical properties of soils, which is well known (Bartlett 1980). As an alternative sample pretreatment, freezing was suggested as the safest way to store soils and to maintain phosphorus information because the reaction between soil and P is virtually stopped at subzero temperatures (Bramley et al. 1992). According to our study, there were no observable changes in the phosphorus fractions induced by freezing the field moist soil except that the Resin-P that was significantly ($P < 0.01$) higher in freezing soils than in moist soils for FS site (Figure 2, Table 3). The changes of P appear to be more pronounced in soils containing higher organic matter in FS soil, and a large fraction of P released after freezing occurs in loosely sorbed P (Figure 2, Table 3). Being similar to air-drying, freezing can also concentrate the soil solution into a smaller volume of liquid and enhanced the sorption of P in soils (Peltovuori and Soinne 2005). In few studies, the solubility of P increased after freezing, and freezing significantly influenced the amount and chemical form of extractable soil phosphorus (Ron and Vaz 1994). On mineral soils, multiple freezing has decreased the concentration of P in soil solution (Bramley et al. 1992, Ron and Vaz 1994). All the results are contradictory and the chemistry of P in freezing soil remains obscure (Peltovuori and Soinne 2005). According to Peltovuori and Soinne (2005), freezing of mineral surface soil does not affect P extractability in water or the P sorption properties of a soil, although it may be more sensitive to soils rich in organic matter. Overall, our results suggested that analysis of fresh soils or freezing the samples is a better way to obtain the phosphorus distribution with respect to field conditions (Bramley et al. 1992, Turner and Romero 2009).

It is shown that air-drying significantly changed the phosphorus fractions in soils. The labile-P (especially resin-P) and organic-P ($NaHCO_3$-Po, NaOH-Po and Con.HCl-Po) significantly increased at the expense of NaOH-Pi and occlude-P (Dil. HCl-P and Con.HCl-Pi) following air-drying the moist soil (Figure 2, Table 3). The resin-P content increased by 30.8% and 120.5% for SS and FS soils, respectively. On the basis of our studies, a more substantial increase in the most labile forms of P would have been expected in the high-TP, high-organic matter soils than in the low-TP, low-organic matter soils (Figure 2, Table 3). Large increases in the concentration of P in water extracts or soil solution have been reported in soils subjected to drying and rewetting (Turner et al. 2003). The published literatures mainly attributed the changes in P solubility to the release of microbial compounds by lysis on rewetting or the disruption of organic matter by the chemical and physical processes involved in drying (Bartlett 1980, Sparling et al. 1985, Turner and Haygarth 2001). Air-drying and rewetting were shown to kill up to ca.70% of the total microbial biomass in soils (Blackwell et al. 2010), and the dead biomass can be a significant source of soluble P. When the soil is air-dried, the water would be released from the microbial biomass. If it is too extreme, the cell damage, plasmolysis or death will occur (Turner et al. 2003). This resulted in the release of cell content of which P is an important component. Second, soil aggregate disruption and cracking of organic colloids releases increase the extractability of the non-biomass soil organic matter and exposes new soil surfaces for microbial attack (Wu and Brookes 2005). It also allows these recalcitrant nutrients to be transformed into a more labile nutrient pool contributing to the flush of nutrient mineralization and solubilization (Gordon et al. 2008).

Our results clearly demonstrated that air-drying drive the transformation of P form occlude-P to labile-P and organic-P because air-drying not only

increased the labile-P but also changed the stability of occlude-P. Soil drying is likely to increase as a result of global climate change (IPCC 2007). Soil desiccation can significantly alter soil chemistry, changing abiotic and biotic properties in ways that affect soil P dynamics (Lichtfouse 2010). These changes have important implications for soil phosphorus dynamics and the P exported downstream. In soils, more than half of the extractable P was found in the upper 30 cm in an analysis of global soil data sets averaged across climate zones and vegetation types (Jackson et al. 2000). According to this analysis, P was more accumulated in the surface layer in soils and has the shallowest distribution of the major plant nutrients in soils. The increasing P availability from occlude-P to labile-P induced by air-drying predicted by global warming will accelerated the weathering of stable phosphate minerals and supplied the plant-available P pool in the soil for plant growth. However, they may threaten plant diversity because the availability of soil P has often been found to be negatively correlated with plant diversity in grasslands and natural systems (Hejcman et al. 2007). On the other hand, the increased labile or soluble P can transfer, via leaching, from soil to surface water and subsequently trigger eutrophization events in the near water-bodies. Blackwell show that air-drying and rewetting significantly changed the concentrations of P, especially dissolved forms in the leachate (Blackwell et al. 2009, 2010). The effects on water quality may be increased by irrigation practices and in regions where climate change causes longer dry periods or more frequent cycles of wetting and drying (Turner et al. 2001).

Two soils with contrast organic matter content were sequentially fractionated using a modified Hedley fraction method. The results show that the conservation of moist soils or freezing the samples is a safe way to obtain the phosphorus distribution with respect to field conditions. A rapid transformation of P fractions occurred when the soil was air-dried. Resin-P responds markedly to air-drying treatment because the values increased by 31% for SS soil and by 121% for FS soils. To a lesser extent, the organic-P also increased upon air-drying, although the tendency seem to be more pronounced in high organic matter content soil. With the increase of labile-P and organic-P, the NaOH-Pi and occlude-P (Dil.HCl-P and Con.HCl-Pi) decreased following air-drying moist soil. Our results have an important implication that the soil drying expected by global warming seem to drive the P transformation form occlude-P to labile-P and organic-P and accelerate the weathering of stable P pool in soils.

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