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Short Communication

Potential contribution of lysed bacterial cells to phosphorus solubilisation in two rewetted Australian pasture soils

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Abstract

Soil drying renders considerable amounts of phosphorus soluble upon rewetting, which may be partly derived from lysed microbial cells. Using direct bacterial cell counting in water and tetra-sodium pyrophosphate extracts of two Australian pasture soils, we found that almost all extractable cells were lysed following the rewetting of dry soils. The amounts of phosphorus in the lysed cells corresponded closely to the increases in water-extractable phosphorus following soil drying, suggesting that bacterial cell lysis is a major source of the released phosphorus.

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Soil drying releases considerable amounts of phosphorus to water compared to the equivalent moist soil (Turner and Haygarth, 2001; Turner et al., 2002). The released phosphorus is mainly organic and appears to be at least partly derived from microbial cells that are lysed by the osmotic shock of rewetting (Salema et al., 1982; Kieft et al., 1987). To confirm this, we performed direct bacterial cell counts on water and tetra-sodium pyrophosphate extracts of two Australian pasture soils. Our aim was to test the hypothesis that soil drying and rapid rewetting lyses bacterial cells and contributes to the increase in water-extractable phosphorus.

Two soils were sampled to a depth of 7.5 cm from experimental sites near the Agriculture Victoria Research Institute, Ellinbank, Victoria, Australia: a USDA Haplustalf (clay 29%, total carbon 5.84%, total phosphorus 0.12%, pH 5.0) and a USDA Haplustox (clay 57%, total carbon 9.74%, total phosphorus 0.20%, pH 5.1). Both soils were grazed and received mineral phosphate fertiliser. Soils were sieved (2 mm), re-moistened to 33% gravimetric water content and equilibrated in the dark at 20 °C for 10 d (Brookes et al., 1982). These were designated as 'moist' soils. Subsamples of the moist soils were air-dried for 7 d at 30 °C on shallow metal trays and designated as 'dry' soils.

Water-extractable phosphorus was determined in moist and dry soils by shaking 10 g soil (dry weight) with 40 ml deionised water end-over-end for 1 h. The extracts were centrifuged for 15 min at $10{,}000 \times g$ and filtered through 0.45 μm membranes. Molybdate-reactive phosphorus was determined by flow injection analysis with phosphorus detection at 690 nm (Karlberg and Pacey, 1989). The short contact time between sample and reagent in this procedure ensures that acid-induced hydrolysis of organic and condensed phosphorus compounds in minimal. Total phosphorus was determined by acid digestion (sulphuric–nitric) and molybdate reaction, with phosphorus detection at 880 nm (APHA-AWWA-WPCF, 1998). Molybdate-unreactive phosphorus was estimated as the difference between total phosphorus and molybdate-reactive phosphorus. This fraction includes organic phosphorus and inorganic polyphosphates (Shand et al., 2000).

Bacterial cells were extracted in triplicate from moist and dry soils. Soils (1 g dry weight) were first extracted with deionised water (10 ml) by shaking end-over-end for 1 h. The extracts were centrifuged at $200 \times g$ for 15 min and the supernatants decanted. Extracted cells were fixed by adding 1 ml concentrated formaldehyde (3% final concentration)

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Table 1
Viable bacterial cells extracted sequentially with deionised water and tetra-sodium pyrophosphate from two Australian pasture soils extracted moist (33% gravimetric moisture content) and after air-drying (30 °C for 7 d). Values are means ± standard errors of three replicate extracts

Soil type	Moist soil (number of cells $\times 10^6$)			Dry soil (number of cells $\times 10^6$)		
	Deionised water	Tetra-sodium pyrophosphate	Total	Deionised water	Tetra-sodium pyrophosphate	Total
Haplustalf	226.1 ± 15.6	417.3 ± 11.1	643.4 ± 19.1	8.56 ± 0.71	nd	8.56 ± 0.71
Haplustox	88.5 ± 1.7	158.5 ± 3.5	247.0 ± 3.9	5.04 ± 0.16	nd	5.04 ± 0.16

nd, no countable cells detected.

and 1 ml 10 mM tetra-sodium pyrophosphate (1 mM final concentration). The soil pellets were re-suspended in 5 ml 10 mM tetra-sodium pyrophosphate with 250 μl formaldehyde. After soaking (16 h), the solutions were sonicated for 3 min (Ramsay, 1984), centrifuged for 5 min at 125 × *g* and the supernatants decanted. Centrifugation at forces greater than 200 × *g* causes a significant number of bacterial cells to be lost from solution (Riis et al., 1998). The pellets were extracted twice more using the same procedure after re-suspending in 5 ml of 2 mM tetra-sodium pyrophosphate. The three extracts were pooled and filtered (20 μm).

Direct cell counts of the entire bacterial community were performed in the initial water extracts and the tetra-sodium pyrophosphate extracts of the soil pellet. All extracts were sonicated for 10 s with an ultrasonic probe at full energy output before analysis. Aliquots of the water extracts (100 μl) or tetra-sodium pyrophosphate extracts (5 μl) were diluted to 5 ml with sterile deionised water and bacterial cells were stained by adding three drops of 10 $\mu g\ ml^{-1}$ 4′,6-diamidino-2-phenylindole (DAPI) and incubating for 2 min. The stained cells were filtered with gentle pressure (0.5 $kg\ m^{-2}$) onto Irgalan-black stained 0.2 μm polycarbonate filters and counted using UV-excitation epifluorescent microscopy (Olympus BH-2 microscope, HBO-100 epifluorescence illuminator, SPlan Apo 100× oil immersion objective, exciter-barrier filter sets for UV (334–365 nm) and blue (435–490 nm) excitation). Cells were counted from between 10 and 30 random fields with a minimum of 10 cells per field and are expressed relative to dry weight of soil. The phosphorus content of the extracted bacterial cells was calculated by assuming the average volume (0.1 μm^3) and phosphorus content (2.44%) of soil bacteria (Anderson and Domsch, 1980; Bakken, 1997).

Numerous bacterial cells were present in the initial water-extracts and tetra-sodium pyrophosphate extracts of moist soils (Table 1). In direct contrast, the water extracts of dry soils contained only small numbers of bacterial cells, whilst the tetra-sodium pyrophosphate extracts contained no countable cells. Thus, almost all the viable cells extractable from moist soils were lysed by the soil drying and rewetting procedure.

The calculated amounts of phosphorus in the extracted bacterial cells are presented in Table 2. The difference between moist and dry soils represents the potential contribution of bacterial lysis to the increase in water-extractable phosphorus following soil drying (Table 3). This was equivalent to 88 and 137% of the increase in

Table 2
Calculated phosphorus contents of bacterial cells extracted sequentially with deionised water and tetra-sodium pyrophosphate from two Australian pasture soils extracted moist (33% gravimetric moisture content) and after air-drying (30 °C for 7 d). Values are means ± standard errors of three replicate extracts

Soil type	Moist soil ($\mu g\ P\ g^{-1}$ soil)			Dry soil ($\mu g\ P\ g^{-1}$ soil)		
	Deionised water	Tetra-sodium pyrophosphate	Total	Deionised water	Tetra-sodium pyrophosphate	Total
Haplustalf	0.552 ± 0.038	1.018 ± 0.027	1.570 ± 0.047	0.021 ± 0.002	–	0.021 ± 0.002
Haplustox	0.216 ± 0.004	0.378 ± 0.009	0.603 ± 0.010	0.012 ± < 0.001	–	0.012 ± < 0.001

Table 3
Increases in the amounts of phosphorus fractions extracted by water following the air-drying of Australian pasture soils (data from Turner et al. (2002)) and the potential bacterial contribution (Table 2). Values are means ± standard errors of three replicate extracts

Soil type	Phosphorus released to water following soil drying ($\mu g\ P\ g^{-1}$ soil)			Potential contribution from lysed bacterial cells	
	Total P	Reactive P	Unreactive P	($\mu g\ P\ g^{-1}$ soil)	%
Haplustalf	1.76 ± 0.05	0.59 ± 0.08	1.17 ± 0.10	1.549 ± 0.047	88
Haplustox	0.43 ± 0.06	−0.20 ± 0.01	0.62 ± 0.06	0.591 ± 0.006	137[a]

[a] Potential contribution of 95% when calculated on the basis of released molybdate-unreactive phosphorus alone.

water-extractable total phosphorus from the two soils. The latter value may be an overestimate, because molybdate-reactive phosphorus decreased in the Haplustox following drying, almost certainly due to an increase in the orthophosphate sorption capacity of this high-phosphorus fixing soil following drying (Haynes and Swift, 1985). However, when calculated on the basis of molybdate-unreactive phosphorus alone, the proportional contribution of lysed bacterial cells was 95% (Table 3). This is not unexpected, because most bacterial phosphorus is organic phosphorus in the form of nucleic acids and phospholipids (Webley and Jones, 1971). The large increases in inorganic phosphorus that occur following chloroform fumigation are due to the enzymatic hydrolysis of the cellular organic phosphorus during the 24 h incubation rather than the release of microbial inorganic phosphorus (Brookes et al., 1982).

The results strongly suggest that lysed bacterial cells were the source of much of the increase in water-extractable organic phosphorus following soil drying. Soil drying and rapid rewetting can kill up to 58% of the total microbial biomass (Kieft et al., 1987; Van Gestel et al., 1993), which represents a substantial pool of phosphorus. Bacterial cell death can occur during both drying and rehydration, but our experimental design did not allow differentiation between these processes. However, cell lysis is likely to be induced by osmotic shock upon the rapid rewetting of desiccated cells rather than by soil drying per se (Salema et al., 1982). Thus, cells remain relatively intact during mild drying, but are lysed upon water extraction, resulting in the rapid release of intracellular phosphorus to solution. It should be noted that extraction with dilute salt solutions, such as 10 mM $CaCl_2$, is unlikely to induce sufficient osmotic shock and cell lysis compared to extraction with deionised water, thus reducing the apparent contribution of cell lysis to nutrient solubilisation (Appel, 1998).

Tetra-sodium pyrophosphate almost certainly extracted only a small proportion of the soil bacteria, because most soils contain around 3×10^9 bacterial cells (Russell, 1988). This suggests that only cells located on the surfaces of aggregates were extracted, probably dominated by fast-growing gram-negative species. Slower growing gram-positive bacteria are more susceptible to drying due to their rigid wall structures, but are protected from all but the most extreme drying events by their location within soil aggregates (Van Gestel et al., 1993). No account was taken of the effect of drying on soil fungi, but the prevalence of these organisms in some soils means that they could make a substantial contribution to the increase in water-extractable phosphorus following soil drying (Sparling, 1985).

In summary, soil drying and rapid rewetting lyses bacterial cells, which may contribute to the observed increases in water-extractable phosphorus following soil drying. Microbial cells may, therefore, be the source of substantial amounts of phosphorus and other nutrients transferred to watercourses following rainfall onto dry soils.

References

Anderson, J.P.E., Domsch, K.H., 1980. Quantities of plant nutrients in the microbial biomass of selected soils. Soil Science 130, 211–216.

APHA-AWWA-WPCF, 1998. Standard Methods for the Examination of Water and Wastewater, 20th ed, American Public Health Association, Washington DC, USA.

Appel, T., 1998. Non-biomass soil organic N—the substrate for N mineralization flushes following soil drying-rewetting and for organic N rendered $CaCl_2$-extractable upon soil drying. Soil Biology & Biochemistry 30, 1445–1456.

Bakken, L.R., 1997. Culturable and nonculturable bacteria in soil. In: Dirk van Elsas, J., Trevors, J.T., Wellington, E.M.H. (Eds.), Modern Soil Biology, Marcel Dekker, New York, USA, pp. 47–61.

Brookes, P.C., Powlson, D.S., Jenkinson, D.S., 1982. Measurement of microbial biomass phosphorus in soil. Soil Biology & Biochemistry 14, 319–329.

Haynes, R.J., Swift, R.S., 1985. Effects of air-drying on the adsorption and desorption of phosphate and levels of extractable phosphate in a group of New Zealand soils. Geoderma 35, 145–157.

Karlberg, B., Pacey, G.E., 1989. Flow Injection Analysis: A Practical Guide, Elsevier, Amsterdam, The Netherlands.

Kieft, T.L., Soroker, E., Firestone, M.K., 1987. Microbial biomass response to a rapid increase in water potential when dry soil is wetted. Soil Biology & Biochemistry 19, 119–126.

Ramsay, A.J., 1984. Extraction of bacteria from soil: efficiency of shaking or ultrasonication as indicated by direct counts and autoradiography. Soil Biology & Biochemistry 16, 475–481.

Riis, V., Lorbeer, H., Babel, W., 1998. Extraction of microorganisms from soil: evaluation of the efficiency by counting methods and activity measurements. Soil Biology & Biochemistry 30, 1573–1581.

Russell, E.W., 1988. Soil Conditions and Plant Growth, 11th ed, Longman, Harlow, UK.

Salema, M.P., Parker, C.A., Kidby, D.K., Chatel, D.L., Armitage, T.M., 1982. Rupture of nodule bacteria on drying and rehydration. Soil Biology & Biochemistry 14, 15–22.

Shand, C.A., Smith, S., Edwards, A.C., Fraser, A.R., 2000. Distribution of phosphorus in particulate, colloidal and molecular-sized fractions of soil solution. Water Research 34, 1278–1284.

Sparling, G.P., 1985. The soil biomass. In: Vaughan, D., Malcolm, R.E. (Eds.), Soil Organic Matter and Biological Activity, Martinus Nijhoff and Dr W. Junk Publishers, Dordrecht, The Netherlands, pp. 223–262.

Turner, B.L., Haygarth, P.M., 2001. Phosphorus solubilization in rewetted soils. Nature 411, 258.

Turner, B.L., McKelvie, I.D., Haygarth, P.M., 2002. Characterisation of water-extractable soil organic phosphorus by phosphatase hydrolysis. Soil Biology & Biochemistry 34, 27–35.

Van Gestel, M., Merckx, R., Vlassak, K., 1993. Microbial biomass responses to soil drying and rewetting: the fate of fast- and slow-growing microorganisms in soils from different climates. Soil Biology & Biochemistry 25, 109–123.

Webley, D.M., Jones, D., 1971. Biological transformation of microbial residues in soil. In: McLaren, A.D., Skujins, J. (Eds.), Soil Biochemistry, vol. II. Marcel Dekker, New York, pp. 446–485.