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Effects of cover crops on phosphatase activity in a clay arable soil in the UK

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Summary

The effect of five cover crop species (radish, buckwheat, vetch, phacelia and oat) alongside an un-cropped control, on the activity and persistence of soil acid and alkaline phosphatase activity was investigated. There was no effect on alkaline phosphatase activity at the time of cover crop incorporation (March), but by the point of maturation of the following oat cash crop (June) significant differences were detected, with the greatest activity following an oat cover crop. Acid phosphatase activity showed species-related significant differences at both sampling dates, with the magnitude increasing by June. Again, plots following an oat cover crop showed the greatest activity, followed by phacelia. This has shown that soil phosphatase enzymes are affected by the presence of a cover crop, that this effect is apparently species-dependent – and not dependent on the amount of biomass from the cover crop – and that cover crops could be a potential means to enhance soil phosphorus cycling.

Key words: organic phosphorus, phosphate monoester, catch crop, green manure

Introduction

The use of phosphate containing agricultural fertilisers is an inefficient process as phosphorus (P) is rapidly fixed onto soil particles, rendering it unavailable to plants. P is also lost from the field through soil erosion and leaching, causing eutrophication in aquatic ecosystems (Pierzynski *et al.*, 2005). Although plant roots take up orthophosphate ($H_2PO_4^-$ and HPO_4^{2-}), this typically only represents a very small proportion of total soil P. These plant-available reserves are constantly cycled between various pools of soil P of which organic P (P_o) represents approximately 30-80% of the total soil P (Stutter *et al.*, 2012). P_o contains at least one covalent bond to a carbon atom, normally through an oxygen atom ester link (Doolette & Smernijk, 2011), and requires enzymatic hydrolysis to release plant-available phosphate. Phosphatase enzymes are responsible for this process, and are classified into two broad groups *viz.* alkaline phosphatase (optimum *in vitro* activity at pH 11) and acid phosphatase (optimum *in vitro* activity at pH 6.5) (Nannipieri *et al.*, 2011). Alkaline phosphatase is produced by microorganisms, whereas acid phosphatase is produced by both plants and microbes. These extracellular enzymes are deposited in the soil either through cell turnover or are secreted by organisms to utilise phosphate reserves within organic matter (Neal *et al.*, 2017). Soil acid phosphatase concentrations may increase when roots are present, but differentiating between plant and microbial production is problematic (e.g. Spohn & Kuzyakov, 2013).

Including cover crops – unharvested crops grown to protect or improve soil quality – within a crop rotation is increasing in popularity, with it now being a recognised environmental option in order to receive direct payments from the Common Agricultural Policy (Defra, 2017). Whilst much research has been done into the benefits to nitrogen cycling by cover cropping, little is known about effects on P cycling.

Given the range of sources of phosphatase production in soils, we hypothesised that different cover crop species would differentially affect soil phosphatase activity, and that any such effects persist when a following cash crop is grown. We tested these hypotheses via a replicated split cover crop trial grown as part of a typical arable rotation.

Materials and Methods

A cover crop trial was established in August 2016 at the Game & Wildlife Conservation Trust (GWCT) run Allerton Project, Leicestershire, UK (N 052°36'53'' W 00°50'31''). The site is a Denchworth series heavy clay loam, pH 7.5, and part of a commercial arable rotation. It receives regular inputs of inorganic phosphorus fertiliser (254 kg ha^{-1} di ammonium phosphate (46% P_2O_5) last applied autumn 2014), and has a P index of 2- as defined by the Fertiliser Manual (AHDB, 2017). The trial involved 18 plots, each nine metres wide, running the full length of the field. Five cover crop species were sown using a Sumo LDS subsoiler with attached seeder: radish (*Raphanus sativus* L.) at 15 kg ha^{-1}, buckwheat (*Fagopyrum esculentum* Moench) at 32 kg ha^{-1}, vetch (*Vicia sativa* L.) at 30 kg ha^{-1}, phacelia (*Phacelia tanacetafolia* Benth.) at 7 kg ha^{-1} and oats (*Avena strigose* Schreb.) at 40 kg ha^{-1}, along with a bare stubble control left standing from the previous winter wheat crop. These six treatments were replicated three times to give a total of 18 plots.

Cover crops were maintained until termination on 15th March 2017 by a herbicide (glyphosate) spray. However, it should be noted that buckwheat is not frost tolerant and was killed off by frost in November 2016, albeit after good initial establishment. Soil samples were taken on 28th March 2017 before a cash crop of spring oats was drilled on 8th April 2017, and soil samples were taken again on the 27th June 2017.

Soil sampling involved taking cores down to 20cm from each plot. To account for field variability a sampling plan was created to allow for nested analysis of variance. The field was split into five blocks perpendicular to the plots, and a sample was taken randomly from each block within each plot, totalling 90 sampling points. A GPS location was recorded for each sampling point, allowing them to be kept the same for the two sampling dates.

Both acid (assessed at pH 6.5) and alkaline (assessed at pH 11) phosphatase activity was determined using the EnzChek™ phosphatase assay kit (Thermo Fisher E12020) following a modified method of Marx *et al.* (2001). A 0.5 g (dry weight equivalent) sample of soil was added to 50 mL of 100 mM sodium acetate buffer adjusted to pH 6.5 or 11. This was then shaken by hand, and placed on a rocker for 30 minutes to allow dispersion of the soil within the buffer. A 50 µL aliquot of this solution was then added to a black 96-well plate followed by 50 µL of the fluorescent substrate (200 µM 6,8-difluoro-4-methylumbelliferyl phosphate) – added to the wells using a multi pipette – to minimise differences in incubation time between wells. Blank (buffer + soil suspension), negative control (substrate + buffer), quench (fluorescent standard + soil suspension), and reference (fluorescent standard + buffer) treatments were included. The plate was then transferred to a Varioskan™ plate reader (Thermo Fisher Corp.), where it was shaken for 5 seconds, incubated at 30 °C for 30 minutes, and then read at excitation and emission wavelengths of 358 and 455 nm respectively.

Data were converted into units of enzyme activity per mL of soil, where one unit is defined as the amount of enzyme that will hydrolyse 1 µM of *p*-nitrophenyl phosphate per minute at pH 4.8 at 37 °C. This was done by creating a standard curve using a known amount of phosphatase enzyme. Results were checked for normality before using a two-level nested analysis of variance to test for significant differences between cover crop treatments.

Results

Sampling at cover crop termination

No significant difference in soil alkaline phosphatase activity was noted between the cover crop treatments with an overall mean of 0.0703 ± 0.00205 Units mL^{-1} (Fig.1)

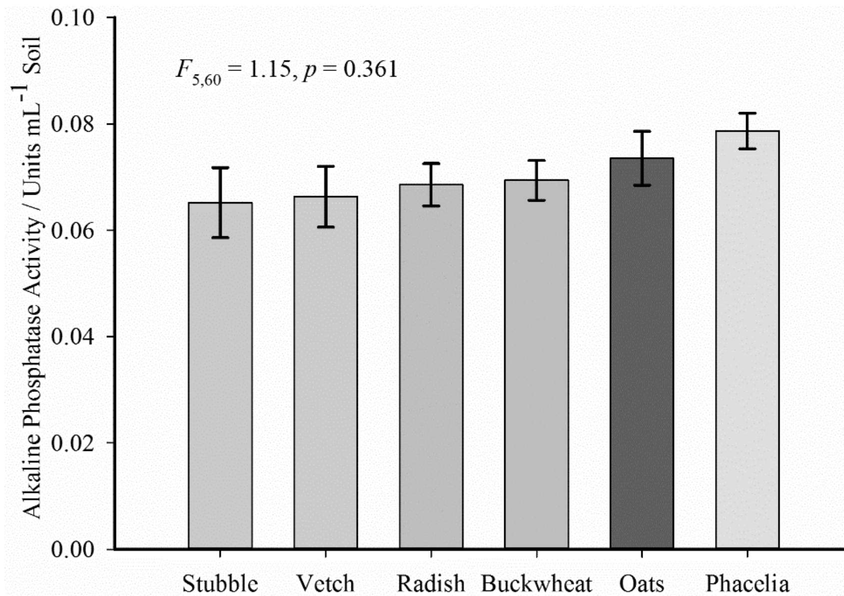

Figure 1*: Soil alkaline phosphatase activity under different cover crop species sampled in March. 1 unit is the amount of enzyme to hydrolyse 1 µmole para-nitrophenyl phosphate per minute.*

Soil acid phosphatase activity was of a similar magnitude to alkaline phosphatase activity, but in this case, there were significant differences between the species of cover crop in the treatments (Fig.2).

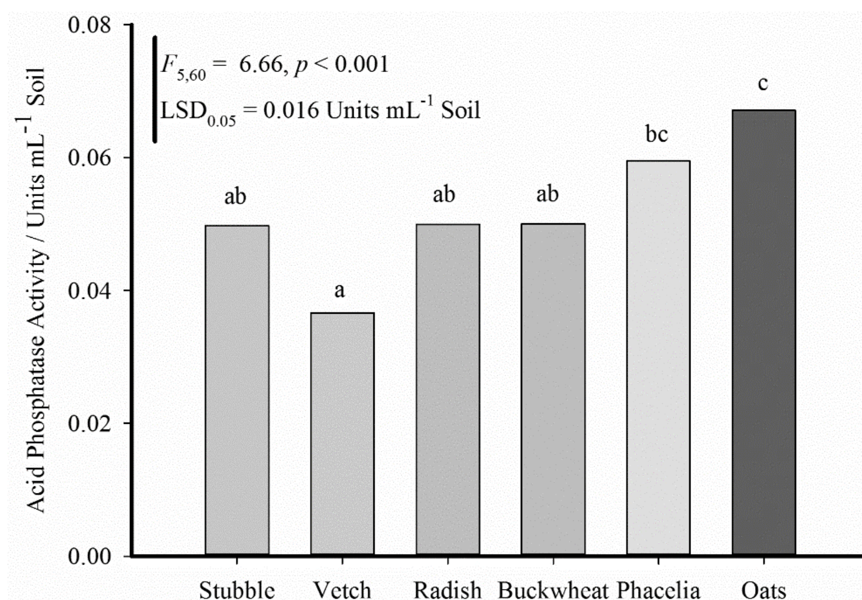

Figure 2*: Soil acid phosphatase activity under different cover crop species sampled in March. 1 unit is the amount of enzyme to hydrolyse 1 µmole para-nitrophenyl phosphate per minute. Species similarly superscripted are not significantly different.*

Sampling at subsequent cash crop maturation

Soil alkaline phosphatase activity sampled on 27th June was greater than when sampled on 28th March, and significant differences due to the cover crop species were also detected (Fig.3). The trend observed for alkaline phosphatase in June was similar to that observed for acid phosphatase (Fig. 2 and 4), with oat cover crop areas showing significantly greater activity than the other four species, and phacelia cover crop areas showing intermediate activity.

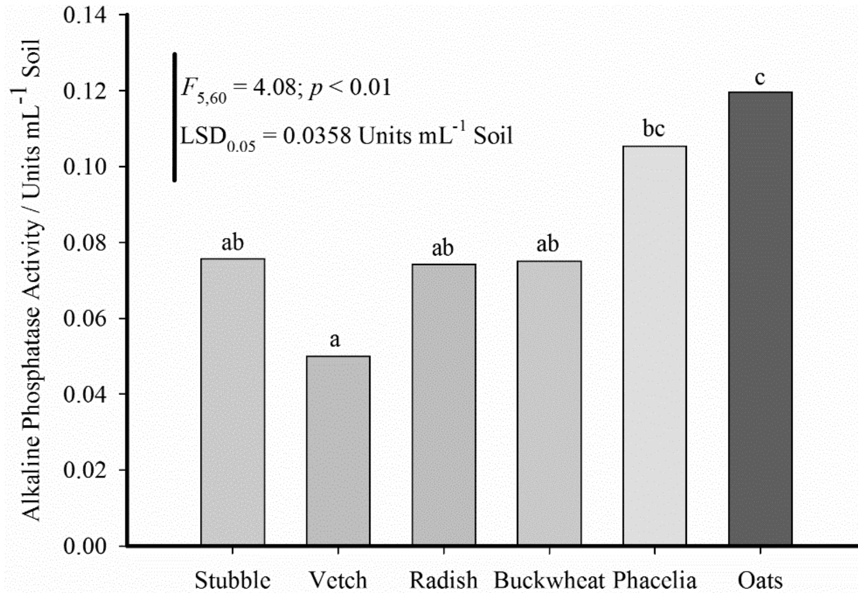

Figure 3*: Soil alkaline phosphatase activity under different cover crop species sampled in June. 1 unit is the amount of enzyme to hydrolyse 1 μmole para-nitrophenyl phosphate per minute. Species similarly superscripted are not significantly different.*

The acid phosphatase activity sampled in June also mirrored that in March, but with all species showing increased activity (Fig. 4). An oat cover crop lead to significantly greater soil acid phosphatase activity than all other treatments.

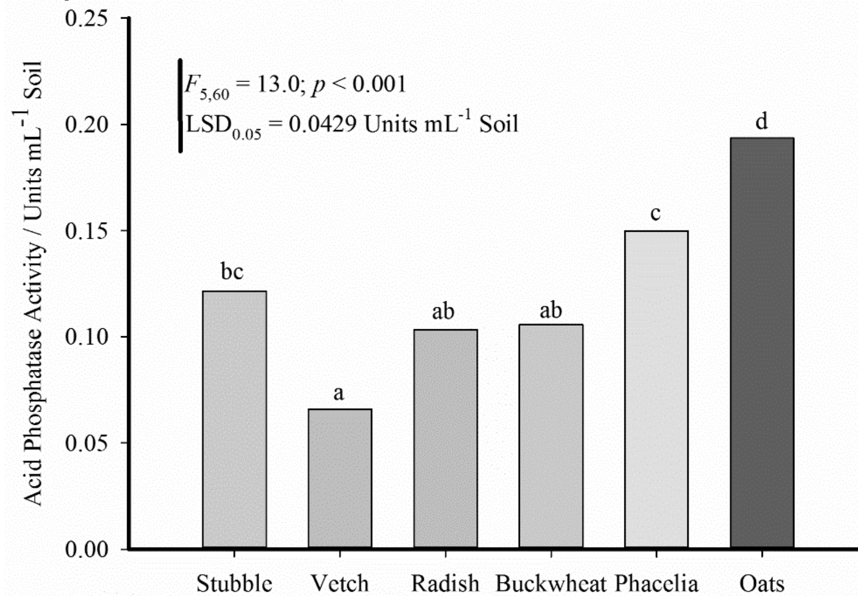

Figure 4*: Soil acid phosphatase activity under different catch crop species sampled in June. 1 unit is the amount of enzyme to hydrolyse 1 μmole para-nitrophenyl phosphate per minute. Species similarly superscripted are not significantly different.*

Discussion

Alkaline phosphatase

It has previously been shown that alkaline phosphatase is not associated with plant roots (Spohn and Kuzyakov, 2013). Therefore, it is unsurprising that growing a cover crop did not affect the activity of this enzyme by the time the cover crop was incorporated. Instead, a background field level was picked up, which did not differ in the presence of the cover crops. By the time of maturation of the cash crop, when the cover crop residue had likely been broken down, differences were detected between the species grown. Activity in plots following an oat cover crop was greater than all but phacelia, which was greater than vetch. Although the growing plants do not directly contribute to the alkaline phosphatase activity, there were detectable legacy effects from the cover crop. Although the biomass varied between cover crop species, it was not detected to be a contributing factor to the soil phosphatase activity. Oat, phacelia and radish cover crops each produced similar levels of biomass, yet showed significant differences between soil phosphatase activity. This indicates that alkaline phosphatase activity in soils can be influenced by growing a cover crop, and by the species of cover crop used.

Acid phosphatase

Differences in the level of soil acid phosphatase activity were detected between cover crop species in both March and June sampled soils, with the species being ranked in the same order, but the activity was greater in June. It is evident that growing an oat or phacelia cover crop affects soil acid phosphatase activity over growing no cover crop, or even growing a crop of vetch, buckwheat or radish. This may be due to just the presence of more plant-fixed carbon in the soil system, as oats and phacelia produced greater above ground biomass than buckwheat and vetch. However, the radish cover crop yielded a similar amount of above ground biomass as phacelia and oat, yet did not affect the soil phosphatase activity over not planting a cover crop. An effect is surely due to the specific species. This may be because more vigorous or branched rooting systems giving a greater root length and so a larger rhizosphere community, which would increase the levels of phosphatase through microbial turnover or exudation. The plants may also be directly exuding phosphatase from roots to access P_o (Tarafdar & Jungk, 1987). A third theory is that this could be due to the plants having a different microbial or fungal community in the rhizosphere, and either encouraging a greater mass of organisms in the rhizosphere, which would then release phosphatase on degradation, or encouraging specific species which utilise phosphatase to access phosphate.

Further work

Although the impact due to field variation has been accounted for in the sampling methodology and statistical analysis, there may still be some differences in the soil samples which are contributing to the results. Further analysis of the samples for nutrient content, particularly the amount of total P, will be informative. The field trial is being continued for another year, although without vetch. This will be sampled more regularly to create a time series of when phosphatase levels fluctuate.

Potential implications for soil P management

This work has shown that using a cover crop can increase the levels of phosphatase activity in the soil, that the magnitude of this effect depends on the species chosen, and that the effect persists into the following crop. Further work needs to be carried out to assess any economical or ecological impact on the agricultural system, but this initial work shows that cover crops can be used to enhance soil P cycling.

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