Geophysical Research Abstracts
Vol. 16, EGU2014-5290, 2014
EGU General Assembly 2014

Applicability and limitations of enzyme addition assays for the characterisation of soil organic phosphorus across a range of soil types

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Solution ^{31}P NMR spectroscopy is a powerful tool for the characterisation and quantification of organic P classes in soil. Potential limitations are due to costs, equipment accessibility and the requirement of relatively large amounts of sample. A recent alternative approach for the quantification of specific organic P classes is the use of substrate-specific phosphohydrolase enzymes which cleave the inorganic orthophosphate from the organic moiety. The released orthophosphate is detectable by colorimetry. Conclusions about the hydrolysed class of organic P can be made based on the comparison of inorganic P concentrations in enzymatically treated and untreated samples.

The aim of this study was to test the applicability of enzyme addition assays for the characterisation of organic P classes on a) NaOH-EDTA extracts, b) soil:water filtrates (0.2 μm) and c) soil:water suspensions. The organic P classes in NaOH-EDTA extracts were also determined by ^{31}P NMR spectroscopy, enabling a comparison between methods. Ten topsoil samples from four continents (five cambisols, two ferralsols, two luvisols and one lixisol) with varying total P content (83 – 1,1560 mg kg^{-1}), pH$_{H2O}$ (4.2 – 8.0) and land management (grassland or cropped land) were analysed. Four different classes of organic P were determined by the enzyme addition assay: 1) monoester like-P (by an acid phosphatase known to hydrolyse simple monoesters, pyrophosphate and ATP), 2) DNA-like P (by a nuclease in combination with an acid phosphatase), 3) inositol phosphate-like P (by a phytase known to hydrolyse all monoester like-P plus myo-inositol hexakisphosphate and scyllo-inositol hexakisphosphate) and 4) enzyme stable-P (enzymatically not hydrolysed organic P forms).

In the ten topsoil samples, NaOH-EDTA-extractable organic P ranged from 6 – 1,115 mg P kg^{-1} soil. Of this, 33 – 92 % was enzyme labile, with inositol phosphate-like P being the largest organic P class in most soils (15 – 51%), followed by monoester-like P (10 – 47%) and DNA-like P (0 – 15%). The four soil organic P classes detected by either ^{31}P NMR spectroscopy or enzyme addition assays were well correlated with each other (R^2 0.93 – 0.99). In soil:water filtrates, 0.1 – 4.1 mg enzyme-labile P kg^{-1} soil were detected, which consisted mainly of inositol phosphate-like P. In some soils, a low absolute amount of water-soluble organic P hindered a more detailed characterisation. In soil:water suspensions, enzyme-labile organic P ranged from 4.3 – 12.6 mg P kg^{-1} soil. However, the enzyme addition assay was only applicable on three soils, since in the other soils i) added enzymes were partly inhibited in soil:water suspensions and ii) the hydrolysis of organic P classes by soil intrinsic enzymes could not be accounted for.

In conclusion, enzyme addition assays appear to be a promising approach for a rapid determination of four main soil organic P classes in NaOH-EDTA extracts. Especially the small amount of required sample size ($<$ 1ml) and the relatively simple instrumentation facilitate a rapid and cheap analysis on these extracts. Application of this method is also possible on soil:water filtrates, but low amounts of organic P may hinder detailed analysis.

Key words: soil organic phosphorus characterisation, enzyme addition assays, ^{31}P NMR spectroscopy, soil suspensions, soil filtrate