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Quantifying and mapping citrate exudation in soil-grown root systems

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The determination of citrate exuded from soil-grown roots is very challenging due to its rapid microbial degradation and mineralization, sorption to the solid soil phase and ongoing release of organic molecules from organic matter breakdown. For this reason, our knowledge about citrate release is mainly based on experiments carried out in hydroponics. Results obtained in hydroponics cannot directly be transferred to soil-plant systems, as hydroponics represents an artificial environment. This study aimed to develop a localization and quantification technique for citrate exuded from soil-grown plant roots, based on diffusive gradients in thin film (DGT). Polyacrylamide gels containing precipitated zirconium hydroxide (ZrOH) were applied to the rhizosphere of soil grown plants, on which citrate is efficiently immobilized, thereby creating a zero sink to sample the citrate exuded from the roots. Citrate was eluted with 1 mL 0.5 mol L^{-1} NaOH from the ZrOH gel and quantified by ion chromatography. ZrOH gel discs were able to bind the citrate contained in 10 mL of 2.77 mg citrate L^{-1} solutions within a 4h uptake period. Elution efficiency was ~89%. ZrOH gel capacity at pH 8 was 200 µg per gel disc and 299 µg per gel disc at pH 4, which is sufficient to act as a zero sink for citrate released from plant roots. As a first exemplary method application, we grew white lupin plants in rhizotrons using a highly phosphorus deficient soil. ZrOH gel sheets were applied for 26 h onto cluster roots for citrate sampling following established DGT protocols. Gels were cut afterwards into 5×5 and 5×2 mm slices for obtaining a citrate exudation map. In both cases we were able to localize and quantify up to 7.89 µg citrate on individual gel slices, as well as to identify longitudinal and lateral citrate gradients around the cluster roots. Moreover, the characterization of ZrOH gels showed its suitability for citrate sampling in terms uptake kinetics and capacity. These results demonstrate that the developed method is suitable for citrate sampling and localization in a non-destructive way from soil-grown plant roots. As it is applicable to soil grown-roots and provides unprecedented spatial resolution, this sampling technique advances the experimental possibilities for researching root exudates considerably. Using suitable binding materials, this approach is also applicable to other carboxylates such as malate or oxalate and other compound classes such as phenolics, flavonolos etc. Furthermore, this technique can be combined with complementary imaging methods for

mapping e.g. nutrients, contaminants, pH or enzyme activity distributions.