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# Soil microorganisms exhibit enzymatic and priming response to root mucilage under drought

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ABSTRACT

Although root mucilage plays a prominent role in soil-plant-water relations, especially under drought, its persistence in soil and its microbial decomposition remain unknown. The aim of this study was to investigate: 1) the effect of soil moisture on mucilage decomposition, 2) the effect of mucilage on enzyme activities, and 3) the effect of mucilage on soil organic matter (SOM) decomposition. We hypothesized that mucilage benefits soil microorganisms by compensating for the detrimental effects of drought. Consequently, low water content was expected to reduce SOM mineralization and enzyme activities only in soil without mucilage. High moisture was predicted to support high microbial activities and therefore rapid decomposition of the mucilage. Two doses of maize root mucilage (40 and 200 μg C $g^{-1}$ soil; C4 plant derived) were added to a C3 soil at optimum moisture (80% WHC) and under drought (30% WHC) to test these hypotheses.

Under optimum moisture conditions, $CO_2$ efflux from soil increased in proportion to mucilage addition. In contrast, there was no effect of mucilage on $CO_2$ efflux under drought. At 80% WHC, mucilage was nearly completely decomposed (98% and 88% for low and high dose, respectively) after 15 days. Drought significantly suppressed mucilage mineralization. Microbial uptake of mucilage C was independent of soil moisture, suggesting that its bioavailability is regulated not by the water content of the whole soil, but by the water within the swollen mucilage. The high mucilage dose increased microbial biomass at both moisture levels compared to the soil without mucilage. Positive priming of soil organic matter decomposition was induced by mucilage at 80% WHC, whereas at 30% WHC, mucilage caused slightly negative priming. Mucilage addition counteracted the decrease of enzyme activities at 30% WHC, and so, stabilized the catalytic activity irrespective of soil moisture content.

We conclude that mucilage provides biofilm-like properties that maintain microbial and exoenzymatic activities, even under drought. The slow decomposition of mucilage in drying soils suggests that mucilage maintains moist conditions around the roots for a long period, supporting beneficial root-microbial interactions at low water availability. This would result in a positive ecological feedback for microbial life in the rhizosphere and enhance nutrient release for roots under water scarcity.

## 1. Introduction

Approximately 805 million people are currently chronically undernourished and this number may increase, as the global population is expected to exceed 9 billion by 2050 (Bishopp and Lynch, 2015). Two major constraints on worldwide crop production are the availability of

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water and nutrients. Many approaches have been suggested to increase plant access to limited soil resources. Roots, in particular, play a prominent role in increasing plant productivity by decreasing water and nutrients limitations. Hence one recently recommended strategy for improving the function and productivity of plants is better management of root zone interactions (Sposito, 2013).

The rhizosphere is defined as the soil volume directly influenced by plant roots (York et al., 2016). A growing body of literature has shown that the properties of rhizosphere soil differ from those of the bulk soil (Carminati et al., 2010; Hinsinger et al., 2009) and that these differences are induced by root activities, for example by rhizodeposition. Rhizodeposits include low molecular weight substances such as amino acids, organic acids, phenols and sugars, as well as higher molecular weight substances including the viscous mixture of exopolysaccharides, proteins and lipids, commonly referred to as mucilage (Chaboud, 1983; Jones et al., 2009). Although mucilage is a major component of rhizodeposits, to date most research has focused on the low molecular weight compounds and their effects on microbial activation and nutrient mobilization and acquisition (Chen et al., 2014; Fischer et al., 2010).

Mucilage is mainly produced at the root tips. It is primarily composed of neutral and acid polysaccharides (94%), proteins (6%) and small amounts of phenolic acids and phospholipids (Bacic et al., 1986; Read et al., 2003; Carminati and Vetterlein, 2013) Mucilage from maize (*Zea mays* L.) has a remarkable ability to swell and absorb water, and fully hydrated mucilage can contain water up to 1000 times its own dry weight (McCully and Boyer, 1997). The properties of mucilage modulate moisture in the rhizosphere (Ahmed et al., 2016; Carminati et al., 2010; Young, 1995), and have been recently speculated to facilitate root water uptake especially from dry soil (Ahmed et al., 2014) and increase plant drought tolerance (Carminati et al., 2016a). However, soil moisture conditions affect not only plant water uptake, but greatly alter microbial activity and physiology (Schimel et al., 2007). Microbially mediated processes such as soil organic matter (SOM) decomposition are therefore dependent on moisture availability (Curiel Yuste et al., 2007), and could be promoted by mucilage in the rhizosphere. Properties of mucilage also resemble those of microbial biofilms that provide advantageous habitats for microorganisms and keep extracellular enzymes close to their producers (Chenu and Roberson, 1996; Flemming and Wingender, 2010; Or et al., 2007). The physical structure of mucilage may therefore make extracellular enzyme production more competitive. Furthermore, mucilage is itself a source of energy and carbon for soil microorganisms (Knee et al., 2001; Jones et al., 2009), affecting both the metabolic and genetic structure of the bacterial community (Benizri et al., 2007).

While it is well known that increased carbon availability can accelerate SOM mineralization ("priming effects" (Kuzyakov and Cheng, 2004)), and that soil moisture directly affects microbial activity (Sommers et al., 1981), the combined effects of mucilage and soil moisture on microbial activity and consequences for SOM decomposition are still unknown. Conversely, the influence of moisture content on mucilage decomposition or stabilization is not known. We hypothesized that the benefits of mucilage for soil microorganisms would compensate for the detrimental effects of drought. We predicted that low water content would reduce SOM mineralization and the activities of SOM-degrading enzymes in bulk soil, but amendment with mucilage would maintain SOM mineralization rates despite limited moisture. On the other hand, we expected that higher water content would support more rapid microbial degradation of the mucilage itself.

Enzyme activities are often assessed in soil by measuring the rate $v$ of an enzymatic reaction at increasing substrate concentrations, [S], and calculating the parameters $V_{max}$, $K_m$ and "catalytic efficiency" $K_a$ defined by the Michaelis-Menten equation (Sanaullah et al., 2016):

$$v = (V_{\max}[S])/(K_m + [S]) \quad (1)$$

$$K_a = \frac{V_{max}}{K_M} \quad (2)$$

For a given enzyme, $V_{max}$ is proportional to the concentration of enzyme present (Palmer and Bonner, 2007). Furthermore, it reflects the rate of enzymatic catalysis at saturating substrate concentration, and therefore does not provide information on how substrate concentration affects enzyme activity. Conversely, $K_m$ is the substrate concentration required for 50% of the $V_{max}$ reaction rate, so it reflects concentration dependence, but not the amount of enzyme present. The "catalytic efficiency" $K_a$ combines both of these measures, and can be interpreted as a measure of enzyme activity at non-saturating substrate concentrations. It therefore provides a better proxy for in-situ enzymatic capabilities in a complex multi-enzyme system such as soil than $V_{max}$ alone.

The aims were therefore: I) to determine the effects of soil water content (optimum and drought conditions) on mucilage decomposition and stabilization and II) to determine the interacting effects of mucilage and soil water content on SOM decomposition and SOM-degrading enzymes. We added two amounts of C4 maize-derived root mucilage: a low dose (10% of microbial biomass C) and a high dose (50% of microbial biomass C) to a C3 soil under optimum (80% of water holding capacity (WHC)) and drought (30% of WHC) conditions. We focussed on a set of enzymes that are implicated in C- and nutrient cycling in soil: beta-glucosidase, cellobiosidase, xylanase, *N*-acetyl-glucosaminidase, acid phosphomonoesterase, tyrosine aminopeptidase and leucine aminopeptidase (Razavi et al., 2016). Given the low protein content of mucilage and the limited proportion of glucose among the component sugar monomers (Bacic et al., 1986) we did not expect mucilage to be a direct substrate or inducer of enzyme expression, but instead to have a general influence on the activities of the enzyme-producing microorganisms.

## 2. Materials and methods

### 2.1. Site description and soil sampling

The site descriptions and soil properties are provided in detail by Sanaullah et al. (2016). We collected the soil samples from the top 25 cm of an arable Haplic Luvisol located in the north-west of Goettingen, Germany. The loamy soil had 7% sand, 87% silt, 6% clay, with pH of 6.0. The total organic carbon (C) and total nitrogen (N) contents were 12.6 and 1.3 g $kg^{-1}$ soil, respectively. Cation exchange capacity was 107.8 $cmol_c$/kg and the soil had a water holding capacity of 30% dry weight. Due to the long-term (> 15 years) C3 vegetation on this field, the $\delta^{13}C$ value of the soil was $-27.4 \pm 0.1$‰. After sampling, the soil was mixed and sieved (< 2 mm) for the incubation experiment.

### 2.2. Mucilage collection

Root mucilage was collected from 8-week-old maize. To this end, we collected nodal roots of *Zea mays* plants growing in the field, from the second and third nodes above the soil. The roots were 2–5 cm long. The distal ends (1–2 cm) of these roots were cut off, placed in water in sealed vials and returned to the laboratory. The hydrated mucilage was removed with fine forceps. The mucilage was applied to the soil immediately after collection. A subsample of mucilage was frozen, freeze-dried and analyzed for isotopic composition, as well as C and N contents. The total organic carbon content of the mucilage was $1.42 \pm 0.02$ g $l^{-1}$ with $\delta^{13}C$ value of $-11.5 \pm 0.1$‰. The C/N ratio of the mucilage was 54.7.

### 2.3. Incubation experiment

The incubation experiment was carried out in 100 mL air-tight jars containing 30 g of soil. Mucilage was added to soil in two doses: a low dose of mucilage (40 µg C $g^{-1}$ soil, equivalent to 10% of microbial