

## Short communication

## The effect of a quorum-quenching enzyme on leaf litter decomposition

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## ABSTRACT

There is much interest in the role of microbial community composition in determining ecosystem processes but the role of interactions within these communities has often been underrepresented. One interaction within microbial communities is quorum-sensing, a type of density-dependent cell-to-cell communication. Here we examine the potential of quorum-sensing effects on litter decomposition in two communities (Forest and Meadow) by adding an enzyme that reduces quorum-sensing. The source of the community and quorum-sensing interacted to determine cumulative litter decomposition. Within the meadow community, enzyme additions caused no effect on decomposition, but in the forest community, inhibition of quorum-sensing resulted in a 14–17% decrease in decomposition. We suggest that quorum-sensing, a community interaction, may be an important determinant of litter decomposition in forested systems.

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Research into the role that microbial community composition plays in determining ecosystem function reveals that composition affects litter decomposition rates (Reed and Martiny, 2007; Schimel and Schaeffer, 2012). One mechanism underlying these composition effects may relate to how the presence/absence of taxa affects the strength of interactions between the constituent taxa. Interactions between species alter ecosystem function (Bonkowski, 2004; Wilmers et al., 2012; Hawlena et al., 2012) but their potential effects within soil microbial communities are often difficult to discern, given high taxon diversity and the opaque soil habitat.

One of the many interactions occurring within microbial communities is quorum-sensing. This density-dependent form of cell-to-cell communication is involved in bacterial group behavior (Case et al., 2008). This behavior includes many ecologically relevant processes, like biofilm formation, extracellular enzyme production, and expression of nutrient acquisition strategies (Hmelo et al., 2011; Van Mooy et al., 2012). The presence of acylated homoserine lactones (AHLs), autoinducers involved in quorum-sensing by Gram negative bacteria, have been detected in bulk and rhizosphere soil, and the litter layer (DeAngelis et al., 2008; Burmølle et al., 2005). The presence of AHLs in litters and soil, and their role in functional trait expression through quorum-sensing,

suggests that quorum-sensing in soil bacterial communities may influence the rate of ecosystem processes controlled by microorganisms. Yet, the role of quorum-sensing in terrestrial ecosystem processes has not been directly shown, despite the importance of quorum-sensing in the functioning of marine systems (Burmølle et al., 2005; Addiscott, 2010).

We examine the potential role of quorum-sensing in two soil microbial communities with regard to leaf litter decomposition, an ecosystem process fundamental in nutrient cycling. We inoculated a sterilized, mixed-deciduous leaf litter (1 g) with 0.25 g of soil from the same forest ( $n = 5$ ) where the litter was collected or an adjacent meadow ( $n = 5$ ). Both sites were less than 200 m from each other. The forest was primarily composed of *Quercus* sp, *Acer* sp, and *Carya* sp and the meadow was composed primarily of *Poa* sp with a mixture of perennial herbs. To inhibit quorum-sensing we added porcine kidney acylase (EC 3.5.1.14), a quorum-quenching enzyme (Yeon et al., 2009), which makes AHL inoperable as an autoinducer. We had two controls. One was a denatured acylase treatment and the other was a water only treatment. Acylase was denatured by heating it to 85 °C for 1 h and this treatment served as a “positive” control for the addition of carbon and nutrients that comprise the enzyme. Water-only addition served as a “negative” control. Both acylase treatments were amended as a 1-mL solution at 0.01 mg g<sup>-1</sup> dry wt litterweek<sup>-1</sup>; water was added of equivalent volume. The addition rate is similar to that used for biofilm

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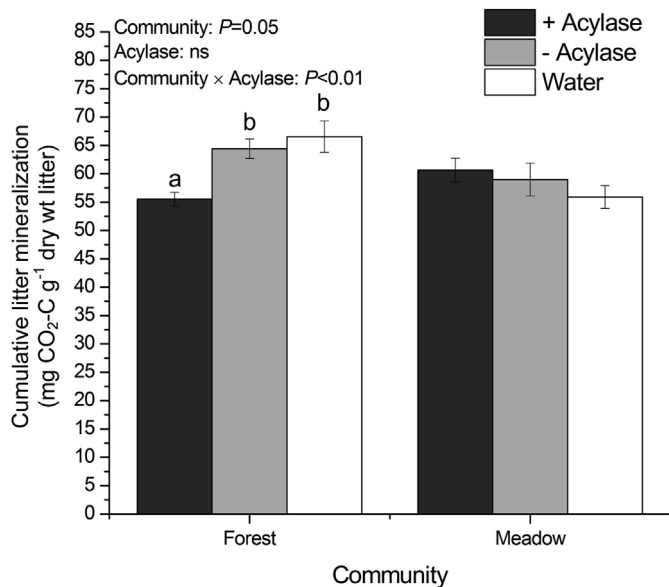
inhibition (Yeon et al., 2009). All microcosms were maintained at 65% water holding capacity after additions.

Litter decomposition was assessed following Strickland et al. (2009). Briefly, the headspace of each microcosm was flushed with CO<sub>2</sub>-free air, incubated at 20 °C for 24 h, and a headspace sample run on an infrared gas analyzer (Li-Cor Biosciences, Lincoln, NE, USA, Model: LI-7000) to determine CO<sub>2</sub> flux. Mineralization rates were measured across 279 days. Cumulative mineralization, calculated by integration, was analyzed using ANOVA, where the independent variables were microbial community (forest, meadow) and acylase treatment (active, denatured, water). All analyses were conducted using R (<http://cran.r-project.org/>).

Microbial community source (forest vs. meadow) interacted with the acylase treatments ( $F_{2,24} = 6.90$ ;  $P < 0.01$ ; Fig. 1) where, for the meadow community, acylase additions had no effect on litter decomposition ( $F_{2,12} = 1.05$ ;  $P = 0.38$ ) but in the forest community they caused a 14% and 17% relative decrease in cumulative mineralization when compared to treatments receiving denatured acylase or water, respectively ( $F_{2,12} = 9.60$ ;  $P < 0.01$ ). Additions of water and denatured acylase to the forest community had statistically similar cumulative litter mineralization (Fig. 1), suggesting that the addition of C and nutrients in the acylase itself had no effect on decomposition.

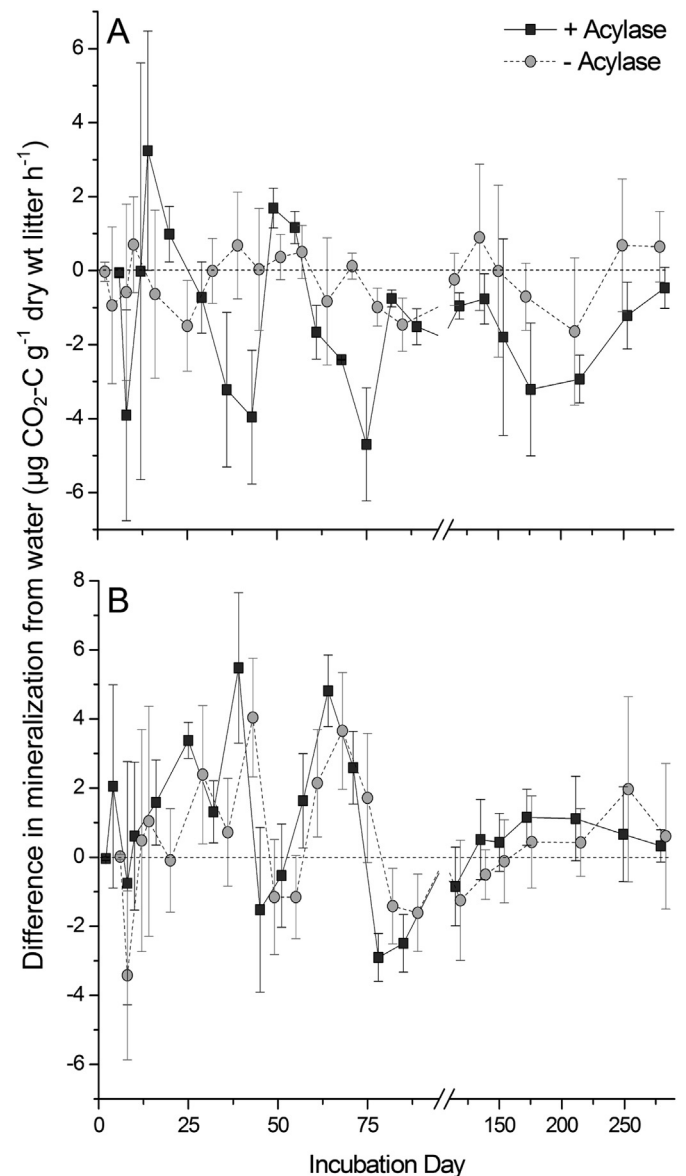
Our results suggest that the effects of quorum-sensing on litter decomposition rates may be of similar magnitude to effects of community composition. That is, the magnitude of the acylase addition effect within the forest community was similar to differences in cumulative mineralization between the communities ( $F_{1,24} = 4.15$ ;  $P = 0.05$ ). For example, in the water only treatments, meadow communities had 16% less decomposition than forest communities (Fig. 1). However, this may be community dependent.

Most interesting in our data is the fact active-acylase addition inhibited decomposition in the forest but not meadow community. This different response may result from the greater abundance of Gram- vs. Gram+ bacteria in the forest vs. meadow community. Phospholipid fatty acid assessments (following Findlay and Dobbs



**Fig. 1.** Cumulative litter mineralized during the 279-day incubation inoculated with either forest or meadow community soil. A significant acylase treatment  $\times$  community interaction occurred because of effects of acylase treatments within the forest but not the meadow community. Within the forest an average 15% decline in litter mineralization was observed for the treatments receiving active acylase, a quorum-sensing inhibitor, compared to the other two treatments. Bars represent means  $\pm$  1 S.E. and letters indicate significant differences between bars.

(1993)) of the field soil from both sites showed that the microbial communities differed: the ratio of Gram-:Gram+ was 1.90 in the forest but 1.51 in the meadow. This is potentially important because AHLs, the autoinducer inhibited by acylase, are produced primarily by Gram- bacteria (Case et al., 2008). Additionally, Gram- bacteria are important producers of extracellular enzymes, such as chitinases and  $\beta$ -N-acetyl-glucosaminidase (Zimmerman et al., 2013). Thus, quorum-sensing in forests may be dominated by AHLs leading to a stronger effect of acylase in this community. Another potential reason for this effect may be due to the fact that only forest litter was assayed. That is, the forest community and the forest litter share a common history (i.e. home-field-advantage; HFA [Gholz et al., 2000; Strickland et al., 2009]). Quorum-sensing, and the processes that it instigates, may be a mechanism underlying HFA. Specifically, quorum-sensing may be adaptive in forest soil communities because forest litter is thought to be a more nutrient poor environment than herbaceous litter, and facilitative interactions



**Fig. 2.** Difference in litter mineralization rates from water for the active and inactive acylase treatments across time compared to the water only control (represented by the dotted line) for the forest community (A) and the meadow community (B), points represent mean  $\pm$  95% C.I. Also note that points were offset and a break was inserted in the x-axis to aid in interpretation.

such as quorum-sensing are considered most important in harsher environments (Michalet et al., 2006). In support of this inference that facilitative interactions such as quorum-sensing are more important in harsher environments, acylase inhibited decomposition consistently in later vs. initial stages of decomposition for the forest community when compared to the water-only control (Fig. 2). Overall, 14 time points fell below the mean of the water treatment for the active acylase treatment compared to only 3 time points for the inactive acylase treatment, most of which occurred later in decomposition. This is likely because there are many labile compounds present at the start of decomposition but in later stages more recalcitrant compounds dominate (Strickland et al., 2009). Our data therefore suggest that quorum-sensing may be an important interaction in communities associated with more recalcitrant litter and as litter ages, and like other facilitative interactions its importance increases with environmental stress (Michalet et al., 2006). However, whether this is generalizable across multiple communities and litter types is unknown and future research should explore the generality of quorum-sensing.

Here, using an acylase inhibitor and microcosm approach, we demonstrate the potential role of quorum-sensing in the ecosystem process of litter decomposition. Research is required to evaluate how quorum-sensing affects litter decomposition rates *in situ*. Our results show that interactions within microbial communities may be important drivers of ecosystem function.

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