Discussion

The usage of organic amendments provides a sustainable solution for agronomic productivity while minimizing disturbances to natural systems(Cambardella et al., 2015; Tejada et al., 2009). To provide an understanding of the impacts of varying organic amendments have on the bacterial community and nutrient cycling in the soil. We selected amendments for this study to represent a range of C:N ratios, with composted manure residues representing a higher C:N ratio and alfalfa residues representing a lower C:N ratio and a mixture of the two representing a neutral C:N ratio. As expected, these ratios resulted in contrasting patterns of nitrogen mineralization when applied to soils. Specifically, we observed that alfalfa amended soils had a significantly higher release of mineral nitrogen due to mineralization than compost, mix, and no amendment reference microcosms. This mineralization response of the alfalfa amendment is due to its low C: N ratio and agrees with previous studies and suggests that in this system, a C:N ratio of 25 is indicative of a balance between N mineralization and immobilization (Kaleeem Abbasi et al., 2015; Kumar and Goh, 2003; Schimel, 2004). As demonstrated by the rate of mineralization in mix amended samples compared to the reference, with mix amended samples not significantly different from the reference on day 49 and within five ppm of the reference for the duration of the incubation.

  The chemical composition of the amendments provides some insight into the nutrients available to the soil microbial communities during these times. We expect that the alfalfa amendment, high in nitrogen, would initially contain complex molecules like cellulose, hemicellulose, and proteins and would require initial decomposition and break down before organic nitrogen would be made available (Andresen et al., 2016; Nguyen et al., 2019). Supporting this expectation, we observed that nitrogen availability within the early response of alfalfa-amended soils was relatively low, under around ten ppm from days 7 to 21, before increasing significantly to 35-40 ppm by day 97. Similar compounds are likely to be present in the compost. However, the nitrogen content was sufficiently low that the inorganic N concentration was below five ppm for the initial incubation period, before dropping to 0 ppm by day 21 and not increasing again until day 97, ending at 5-10 ppm, clearly showing immobilization of inorganic N as the microbial community tries to decompose the high C content.

The microbial community of the incubated microcosms was more strongly influenced by time than by amendment type. While contributions to community variation from treatment, C:N ratio, and inorganic N concentration are significant among the environmental and explanatory variables, time remains the most influential driver. When viewed as a whole, the amendments appear to have a limited effect on the relative abundance at the phyla level. However, when we look at a more granular scale and in temporal groups, we can identify phylogenic associations with specific amendment response groups. Differential abundance analysis using normalized counts enabled the identification of OTUs responding to amendment by comparing amended samples to no amendment reference samples. Regardless of the amendment, we observed two distinct temporal groupings of samples throughout our experiment. We defined these as early (up to day 21 after amendment) and late response (days 35-97) based on the observed soil microbial communities.

  Generally, we expect that the early responders represent an ability to facilitate organic amendment usage through the initial conversion of nitrogen and also carbon for plant and microbial growth. The identification of the presence of Proteobacteria in early responders in all amendments is consistent with previous studies showing cellulase enzyme production from Pseudomonas spp. (Bakare and Adewale, 2005), and its enrichment in response to high cellulose and hemicellulose content in alfalfa hay. This finding may contribute to our understanding to how N is mineralized from plant residues (Peterson and Russelle, 1991).

  Some notable early responders identified are associated with specific amendments, several OTUs responding to amendments are only observed in microcosms receiving alfalfa or compost. The response of species in the early alfalfa, not responding in compost, is consistent with our hypothesis that there are specific communities that may be necessary for optimizing nutrient cycling in organic amendments, depending on the characteristics of the amendment. We also observed sequences related to legume symbionts from the genus Rhizobium and the genus Burkholderia in the early response groups, which are associated with nitrogen cycling (Bottomley et al., 2012). In general, we expect that these early responders represent an ability to facilitate organic amendment usage through the initial conversion of nitrogen and carbon for microbial growth.

  One specific OTU identified to be significantly more abundant in all amendments and response groups when compared to reference soils, OTU 00847. OTU 00847 is a member of the phyla Planctomycetes and is unclassified at the genus level. We also identified six other OTUs associated with Planctomycetes that were significant late responders under compost and alfalfa amendments.  Further, Planctomycetes respond to extracellular polysaccharide production by other community members, which may explain OTU 00847’s ubiquitous response (Wang et al., 2015). It is possible that instead of responding to the amendment itself, members of this OTU may respond to other bacteria exuding EPS during the incubation.

We evaluated the genomic similarity of OTUs associated with early and late responders to understand if there exist phylogenetic patterns in the responders broadly across incubations. For specific amendments, we do observe phylogenetic patterns among early and late responders. The phylogenetic conservation of groups of significant OTUs in early and late response periods provides further support that the identified specific microbial membership is responding to nutrient availability. Previous studies have also found that the functional ability to degrade available compounds is often phylogenetically conserved (Morrissey et al., 2016).

  In conclusion, our study suggests that there are predictable soil microbial members that respond to specific amendments. These microbes represent up to one percent of the microbial community in a given treatment response group. Except for a few OTUs, these microbes originate from soil, indicating the importance of a diverse soil microbiome in responding to organic matter incorporation. Utilizing soils from established organic alfalfa plots likely contributed to the diversity of the microcosms during the incubation, organic soils consistently harbor a more diverse bacterial community than mineral fertilized soils (Bakker et al., 2018). Further work utilizing different amendments or starting soils could inform our understanding of how the native communities of less diverse soils respond to amendments. Soil microbial diversity and management practices are associated with one another, and our study extends this to organic amendment management (Birkhofer et al., 2008). Long-term management practices have been observed to impact microbial diversity, with distinct microbial membership identified in long-term organic versus conventional farming soils (Hartmann et al., 2015). The OTUs we have identified responding to amendment a within soil microbial communities identified within this study highlights opportunities for research for both alfalfa and compost during the initial and late stages of nitrogen availability.

  Overall, this highlights the need and opportunity for characterizing the microbial activity for several organic amendments and management practices. We conclude that merely applying amendments on a total nitrogen basis does not guarantee the same amount of inorganic nitrogen available to plants. The varying mineralization response shows this, despite all amendments supplying the same amount of total N. Further, the unique response of OTUs to specific amendments highlights the importance of a diverse microbial community for decomposition and may hint at the role of complex inputs in supporting a diverse microbial community.

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