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). The amendments selected for this study were chosen because they are common????? organic amendments and also because they 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. In this study, we aimed to better understand the microbial dynamics in response to these varying soil amendments. To better characterize these dynamics, we first had to understand the availability of carbon and nitrogen within the amendments and subsequently in the soil microcosms after amendments.

The varying availability of carbon and nitrogen within these amendments resulted in contrasting patterns of nitrogen mineralization when applied to soils. Specifically, we observed that alfalfa amended soils had significantly higher release of mineral nitrogen than any other amended or unamended soil microcosms. This mineralization response of the alfalfa amendment is due to its low C: N ratio and is consistent with previous that suggest a C:N ratio of 25 balances between N mineralization and immobilization (Kaleeem Abbasi et al., 2015; Kumar and Goh, 2003; Schimel, 2004). In the mixed amendment, where the C:N ratio was measured as XXX, we observed that the rate of mineralization was generally not significantly different from no amendment reference soils.

  We also considered that the chemical composition of the amendments provide varying nutrients that are available to soil microbial communities. In the alfalfa amendment, which is relatively high in nitrogen compared to other amendments, we expect initially complex molecules like cellulose, hemicellulose, and proteins, requiring initial decomposition and break down before organic nitrogen would be made available (Andresen et al., 2016; Nguyen et al., 2019). In support of this expectation, we observed that inorganic nitrogen availability within the early response of alfalfa-amended soils were relatively low (under around ten ppm from days 7 to 21, before increasing significantly to 35-40 ppm by day 97). In compost, we expect similar chemical compounds to be present but at a lower abundance based on C:N ratios of amendments. In compost, we observed trends in inorganic N availability that suggest low availability of inorganic N and suggesting that immobilization of inorganic N may be the dominant as the microbial community decomposes the high C content in compost. Next, we considered the dynamics of the microcosm soil communities under these conditions.

Generally, we observed predictable shifts in the microbial communities within amended microcosms. These shifts were influenced by amendment type, C:N ratio, and inorganic N concentration but time since amendment was the most influential variable to explain microbial community composition. . 21 daysGenerally, early responding samples also can be characterized as low observed inorganic nitrogen(<15 ppm) and increasing microbial biomass. These results suggest that early response may be indicative of N incorporation into microbial cells (immobilization), while late response may suggest more inorganic N availability (mineralization conditions).

[Before we go into the specific amendments, we need some paragraph of generally who the early and late responding phyla – generally over both amendments are. You’ve pointed out that the main story here is time, so we need to give some thought of who is responding in time and whether it is the same guys or different? Are these abundant or small proportions of total community?s Across amendments are theyt he same or different?]. 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.] 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. 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).    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.

In comparing the microbial communities in classified early and late samples between amendments, the amendments appear to have a limited effect on the relative abundance at the phyla level. However, at a more granular scale, we observe patterns of phylogenetic associations with specific amendment response groups, suggesting that specific microbial membership is responding to nutrient availability. These results are consistent with previous studies finding the functional ability to degrade available compounds is often phylogenetically conserved (Morrissey et al., 2016).   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.

  [Need some text on anything that might or might not be amendment specific – who are they].

  In conclusion, our study indicates that there are predictable patterns of microbial response to organic amendments of soils and that these patterns can be divided into an early and late temporal response. Our identification of these responses can be useful for understanding the nutrient availability in soils in response to amendments. A key observation is that we observe both key early and late bacteria responders to soil amendments. Within our study, we identify that the first major short-term response to amendments is observed in the first three weeks after amendment, followed by a second shift in microbial communities. While amendments do introduce diverse non-native bacteria to the soil, these amended bacteria are not observed to persist in the soil. Both early and late shifts observed in microbial communities due to amendment were observed to originate from the native soil community, most likely in response to available nutrients. Our results of the timing and composition of the microbial response to organic amendments can help to guide management strategies. For example, based on our results, bioaugmentation of microbes through amendments may be challenging, considering that very few (<X%) amended bacteria were observed to be enriched in our microcosms. Also, future experiments can be guided by our consistent observation of a short-term three week initial response to amendments.

Importantly, other studies have also shown soil microbial diversity and management practices are associated with one another, . These studies highlight that….Our study extends this to…. 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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