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Analysis of the nutrient composition, efficacy, and sustainability of bokashi fertilizers

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Executive summary

Three-fourths of the world's people living in extreme poverty rely nearly entirely on agriculture for income, and many cannot produce sufficient food to earn an income or even to feed their families (CGIAR 2014). Present global food production is enough to eliminate hunger, but the food remains largely inaccessible to the hungry (Tscharntke et al. 2012; Chappell and LaValle 2011; FAO 2011). Small farmer agroecological techniques can meet high yields and foster biodiversity (Kremen 2015; De Schutter 2011; Cornia 1985; Barrett, Bellemare, and Hou 2010; De Schutter 2011; Horlings and Marsden 2011; Tscharntke et al. 2012). Global reports argue that within ten years, small farmers can double food production by utilizing these already existing agroecological methods (IAASTD 2009; De Schutter 2010; Altieri, Funes-Monzote, and Petersen 2012). Promoting knowledge-based, small farmer agroecological techniques could increase food accessibility for hungry and poor farmers who cannot support their livelihoods on high agrochemical inputs (Tscharntke et al. 2012).

By using a reductionist approach to contribute to modern ecological knowledge—which tends to be broad but shallow—and understand the intricacies of a traditional agricultural practice, I aim to bridge the gap between knowledge systems to reconcile the advantages of both approaches and generate knowledge both deep and broad. By assessing analytically the properties of bokashi—a traditional fertilizer characterized by the anaerobic and aerobic decomposition of organic matter—I am contributing to an agroecological approach to research by meeting testimonials of bokashi efficacy and economic sustainability with an enhanced understanding of system interactions. Ultimately, this approach seeks to reinforce the value of local knowledge, while assessing implications for global adaptability and contributing to sustainable agroecology and food sovereignty.

Among the advantages of bokashi is that it can be made from a variety of waste inputs, so long as the underlying process of fostering both anaerobic and aerobic microbial processes is maintained. This study explored the nutrient composition and efficacy of bokashi made from either raw or charcoaled rice hulls, or yeast or indigenous microorganism (IMO) starter cultures; the treatment variations were designed based on common bokashi recipes.

A comparison of the nutrient composition during the maturation of bokashi made from different ingredients illustrates the high ammonium and phosphate compositions of fully matured bokashi, regardless of starting ingredients. High ammonium levels were attributed to increased nitrogen mineralization. The supply of bioavailable nitrogen by bokashi was predicted to reduce common nutrient limitations in agricultural systems. Moreover, a controlled study of the effects of bokashi fertilization on the growth of kale and cucumber plants illustrated the heightened growth of crops treated with the nutrient-rich fertilizer. Positive correlations between bokashi ammonium concentrations and crop leaf chlorophyll concentrations, as well as positive correlations between chlorophyll concentrations and plant biomass provide mechanistic insight into how the increased supply of nitrogen by bokashi amendment promoted increased crop growth. Only minimal variation in the effects of the different bokashi types on plant growth were observed, reinforcing the flexibility of bokashi as an amendment that can be made from various ingredients without compromising efficacy.

Ultimately, these findings reinforce the productivity and sustainability of bokashi fertilizers. In terms of productive, bokashi is a nutrient-rich amendment that significantly increases crop growth. In terms of sustainability, bokashi diverts waste streams, such as manure, converting them into a beneficial soil amendment. Furthermore, the flexibility of bokashi fertilizer—characterized by little variation in nutrient composition and effects on plant growth of different types of bokashi—renders it adaptable to various resource availabilities, decreasing the cost of expensive inputs. In other words, farmers can adapt bokashi ingredients based on accessible materials, without compromising the qualities of the fertilizer.

I have assessed analytically the properties of bokashi in an attempt to overcome the challenge of high uncertainty in our understanding of complex ecosystems as a barrier to combining scientific theory and native knowledge (Vandermeer and Perfecto 2013). However, challenges to this approach include incomplete an understanding of the complex biological and chemical interactions underlying the maturation of bokashi, as well as the difficulties with maintaining a bidirectional exchange of knowledge when employing a scientifically reductionist approach.

To overcome these challenges, agroecological research must be done by directly collaborative interdependent science, one in which conventional scientists—those with formal education and training—and civil scientists—those who develop knowledge in various, non-formalized methods—partner as equals in developing more complete knowledge systems (Fortmann 2008; Méndez, Bacon, and Cohen 2013). In this framework, I characterize the progress I have made in this study as a contribution to an early stage of this approach.

The next stage of this interdependent approach involves partnering directly with local farmers to further adapt and study agroecological techniques like bokashi, without erasing their origins and value as traditional agroecological techniques. This agroecological approach could bridge gaps in knowledge systems by broadening deeply explored, but highly locally-relevant, field knowledge, and substantially increasing food production by implementing techniques that already exist and may be adapting (IAASTD 2009; De Schutter 2010; Altieri, Funes-Monzote, and Petersen 2012). Fostering and sustaining this bidirectional exchange of information will be challenging, but no amount of scientific study will promote food sovereignty without equitable and sustained partnerships with the people most immediately involved in cultivating food systems.

Chapter 1

Bokashi as a case study for bridging gaps in knowledge systems

1. Introduction

1.1 Small farmers, big producers

Over one billion people worldwide do not have access to sufficient food, and over two billion are malnourished (Tscharntke et al. 2012; FAO 2011). Most of the world's poorest and hungriest people live in rural environments and depend directly on agriculture for their livelihoods (IAASTD 2009). Three-fourths of the world's people living in extreme poverty rely nearly entirely on agriculture for income, and many cannot produce sufficient food to earn an income or even to feed their families (CGIAR 2014). Adding further to this paradox of hungry farmers (Bacon et al. 2014), peasant and small farmers in developing nations produce most of the food consumed domestically (Altieri, Funes-Monzote, and Petersen 2012; GRAIN 2014): at least 50% and up to 90% of food produced in developing nations is produced by small farmers (GRAIN 2014; IAASTD 2009).

Over 90% of farms globally are small farms,[1] yet these farms account for just under 25% of global agricultural lands (GRAIN 2014). On these tiny fractions of land, estimates reveal that about half of these smallholders utilize a variety of resource-conserving agricultural techniques, many of which evolved locally as agricultural insight was shared across generations (Altieri and Toledo 2011). This knowledge-based agricultural approach requires careful management and intensive labor to foster the long-term ecological health and sustained productivity of these farms without massive inputs (Kremen 2015; Horlings and Marsden 2011). In effect, small farmers that utilize these agroecological techniques cultivate sustainable, biodiverse, and resilient systems.

Present global food production is enough to eliminate hunger, but the food remains largely inaccessible to the hungry (Tscharntke et al. 2012; Chappell and LaValle 2011; FAO 2011). This affirms the fact that intensified industrial agriculture, though productive in yield, has not lead to global increases in food security (Vandermeer and Perfecto 2013;

[1] As defined here, the average size of a small farm is 2.2 acres.

Patel 2010). Rather, new technologies that benefit large scale-commodity agriculture, further disadvantage small farmers who have less access to resources, information, and credit (Altieri 2002). In addition, small farmer agroecological techniques can meet high yields and foster biodiversity (Kremen 2015; De Schutter 2011; Cornia 1985; Barrett, Bellemare, and Hou 2010; De Schutter 2011; Horlings and Marsden 2011; Tscharntke et al. 2012). Global reports argue that within ten years, small farmers can double food production by utilizing these already existing agroecological methods (IAASTD 2009; De Schutter 2010; Altieri, Funes-Monzote, and Petersen 2012). Promoting knowledge-based, small farmer agroecological techniques could increase food accessibility for hungry and poor farmers who cannot support their livelihoods on high agrochemical inputs (Tscharntke et al. 2012).

1.2 Learning from small farmers

In addition to applications in agriculture, the benefits and accuracy of native knowledge have been illustrated and utilized in forestry (Menzies 1994, 2007; Sivaramakrishnan, 1999, Baker and Kusel, 2003), wildlife conservation (Danielson et al., 2005; Goldman, 2007), veterinary medicine (Davis, 1995), and other fields. An appeal of input-based agriculture is its replicability across contexts. Nonetheless, knowledge-based agriculture practices—though specific to the local environments where they were developed—may be replicated in concept. Farmers and ecologists alike may learn from the intergenerational and experiential study of agroecosystems by small farmers. Despite the wealth of accumulated knowledge and innovation underlying smallholder agroecological practices, little attention has been given to protecting or disseminating these techniques (Altieri and Toledo 2011; Koohafkan and Altieri 2011).

Altieri (2002) reasons that this disregard for smallholder innovation stems from a "top-down transfer-of-technology approach" that elevates the value of modern scientific research while ignoring traditional knowledge and the inclusion of local peoples in research. Even in cases when knowledge from marginalized groups are incorporated into larger scientific and political debates by sources generally accepted as credible—such as in scientific publications—Fortmann (2008) points out that the people sourcing the knowledge are still largely excluded from participating directly. This so-called "epistemic injustice" maintains the divide between sources of knowledge and hinders the potential for synergistic collaboration (Fortmann 2008; Fricker 2009).

In contrast, Altieri (2002) calls for a "bottom-up approach" in agricultural research that builds upon local peoples' native knowledge and available resources. While I agree with the intentions of the approach, I find calling it "bottom-up" propagates the very hierarchy of knowledge that we must avoid: one that values scientifically reviewed knowledge above local or experiential knowledge. To emphasize the equal contributions and values of different forms of knowledge, Fortmann (2008) promotes an "interdependent science" approach to improving rural livelihoods, while practicing conservation. In this model, conventional scientists—those with formal education and training—and civil scientists—those who develop knowledge in various, non-formalized methods—must partner as equals in developing more complete knowledge systems (Fortmann 2008; Méndez, Bacon, and Cohen 2013)).

Furthermore, given the generally deep but narrow scope of traditional agricultural knowledge and the broad but shallow character of modern ecological knowledge, practicing interdependent science could combine the knowledge reservoirs into approaches that are both deep and broad (Vandermeer and Perfecto, 2013). In effect, this bidirectional exchange of knowledge between conventional science and experiential application functions within an *agroecological approach*. Agroecological research examines complex ecological relationships and processes, including not just the environmental dimensions, but also the social impacts and economic viability of agricultural systems (Gliessman 1998; Méndez, Bacon, and Cohen 2013).

1.3 Bokashi as a sustainable agroecological technique

To develop an agroecologically sound natural resource management approach that builds on native knowledge, the included techniques must increase farm productivity, prove environmentally and economically sustainable, and be adaptable to various climates and contexts (Altieri 2002). A myriad of practices exist that meet these criteria to variable extents, including the widespread use of compost by small farmers.

The benefits of compost are well-known and the organic amendment is widely used. Compost diverts waste streams as microbial degradation produces a carbon-rich soil amendment. Though various forms of composting exist, the process can fundamentally be described as the controlled breakdown of organic matter into humus by microorganisms (Bernal, Alburquerque, and Moral 2009; Aurora Gomez-Velasco et al. 2014). During composting, largely aerobic microorganisms convert the organic waste into inorganic, plant-available forms (Leconte et al. 2011; Aurora Gomez-Velasco et al. 2014). A diverse community of microorganisms employing various metabolic pathways participate in this mineralization process. Nevertheless, composting is a largely aerobic process that requires regular aeration and hydration. If compost piles are not well homogenized, regions of dominant anaerobic activity slow down waste degradation, create bad odors, and release large amounts of methane, carbon monoxide, sulfides, ammonia, and volatile organic acids (Beffa et al. 1996). Consequently, while compost is a beneficial and resourceful agricultural technique, cited barriers to small farmers utilizing compost include the labor intensity, associated costs, and lack of information on compost quality (Paul et al. 2017).

A similar organic amendment that may be less labor intensive and less costly is *bokashi*, a fertilizer of fermented manure and other waste products. Bokashi is a traditional agroecological technique first developed in East Asia and now utilized extensively across Asia and Latin America. While composting often requires extended maturation times, frequent aeration and hydration, and large spaces, bokashi matures in approximately two weeks and is made in smaller piles for simpler management. The maturation stage of bokashi to fosters beneficial microbial growth, breaks down nutrients to bioaccessible forms, and processes materials so that they no longer attract pests (Nishio 1996). Perhaps this final characteristic granted bokashi its name which translates from Japanese to mean, "obscuring the direct effectiveness" (Nishio 1996).[2] Several studies support the benefits of bokashi on soil fertility and plant growth (Álvarez-Solís et al. 2016; Aurora Gomez-Velasco et al. 2014; França et al. 2016; Lima et al. 2015; Peralta-Antonio et al. 2014; Jaramillo-López, Ramírez, and Pérez-Salicrup 2015; Bautista-Cruz et al. 2014; Boechat, Santos, and Accioly 2013; Aurora Gomez-Velasco et al. 2014).

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[2] I have seen various translations of bokashi into English, including "fermented fertilizer" and "blur."

2. Bokashi: a fertilizer of fermented waste materials

2.1 Bokashi background

Like compost, bokashi is the product of microbial breakdown of organic matter sourced from waste. The main components of bokashi include manure, soil, and carbon-rich agricultural byproducts, such as rice hulls and bran. Unlike compost, bokashi processing largely makes use of the anaerobic process of fermentation. Although bokashi piles must be aerated daily and, consequently, are not completely anaerobically processed, the piles still must also be covered tightly to limit oxygenation. Both aerobic respiration and fermentation are metabolic processes that break down organic compounds by oxidation to generate energy. Oxidation, the loss of electrons, must be coupled with reduction, the gain of electrons. In respiration, the species that gains, or accepts, electrons are oxygens; in fermentation, the electron acceptor must be an organic compound. Anaerobic respiration is another metabolic pathway in which the electron acceptor is anything but oxygen, such as nitrate, sulfate, and carbon dioxide. Although both fermentation and anaerobic respiration proceed without oxygen, they are distinct processes (Jurtshuk 1996).

Studies site that bokashi is generated mostly by lactic fermentation, characterized by the production of lactic acid from the breakdown of glucose (Boechat, Santos, and Accioly 2013; Jurtshuk 1996). Because bokashi piles must be aerated during maturation, bokashi production is characterized by partial anaerobic conditions at the center of the pile and an aerobic environment in the outer layers (Boechat, Santos, and Accioly 2013). This combination of aerobically and anaerobically active organisms promote decomposition and enzymatic fermentation (Lima et al. 2015). These partially anaerobic conditions coupled with energy-rich organic matter allows for the accelerated breakdown of organic matter in bokashi (Álvarez-Solís et al. 2016)

The microbial communities' characteristic of bokashi and compost are similar in that they both include various bacteria, actinomycetes, yeast, molds, and other fungi (Beffa et al. 1996; Ndona et al. 2012). To promote this diverse group of microorganisms and their coexistence, in the early 1980s, Dr. Teruo Higa, professor at Ryukyus University, Okinawa, developed a mix that he called "effective microorganisms" (EM), which included lactic acid bacteria, yeasts, photosynthetic bacteria, actinomycetes, and fungi (Ndona et al. 2012).

Although the EM mix is not required for bokashi production, several studies examining the efficacy of bokashi analyze EM bokashi specifically (Pei-Sheng and Hui-Lian 2002; Boechat, Santos, and Accioly 2013; Lima et al. 2015; França et al. 2016).

Furthermore, EM has become an important component of bokashi *compost*. Bokashi compost provides a way to compost food stuff that are typically excluded from aerobic composting, such as meat and dairy products. This system of anaerobic buckets is different, though similar in the use of fermentation, is different from the bokashi fertilizer previously described as an agroecological technique, and the bokashi of interest in this study.

2.2 Personal experiences

I first learned about bokashi while practicing organic agriculture at the Asian Rural Institute (ARI)[3] in Japan, during the summer of 2016. Bokashi intrigued me as the farmers swore by its efficacy and described how the soil-like amendment was made completely from diverted waste streams and locally available resources: chicken manure, rice husk, rice bran, local microorganisms, and soil. Moreover, my farming mentor explained how the strength of bokashi came not just from its addition of nutrients, but also beneficial microbes that would restore balance to soil microbiome, effectively reducing the risk of plant disease (Abo-Sido 2016, *unpublished*[4]).

Many of the participants shared that bokashi was among the most important things they learned at ARI. Most participants emphasized that the natural fertilizer was made from essentially free resources, thus, possessing the potential to remove the people in their community from an economically and environmentally unsustainable reliance on synthetic fertilizers. One participant from Malawi discussed the benefits of the faster maturation time of bokashi relative to compost, which she hoped would "reduce bad smell and air pollution," from heaps of unfermented manure (Abo-Sido 2016, *unpublished*). Another participant from Ghana performed his own experiment to compare the efficacy of bokashi

[3] At ARI, rural leaders from Asia and Africa—known as participants—train in sustainable, organic agriculture and servant leadership.
[4] Information from my personal journal, entry made on 7/16/2016

relative to other organic amendments (Figure 1), and expressed a noticeable improvement in tomato plant growth upon the addition of bokashi relative to a tomato plant treated with rice husk charcoal alone

Figure 1: An ARI participant explains his experiment comparing the growth of tomato plants treated with (A) just rice husk charcoal, (B) just bokashi, and (C) bokashi, rice husk charcoal, and compost combined. He concluded that amending soils with bokashi, rice husk charcoal, and compost combined improved plant growth the greatest and that rice husk charcoal alone did not supply the plants with sufficient nutrients.

I, too, wanted to test the efficacy of bokashi. Moreover, I pondered the fertilizer's adaptability given that the ARI participants came from remote villages in countries across Asia and Africa, with climates and available resources that differed from that of and those available in Japan. Nevertheless, participants expressed that while learning the principles underlying bokashi, they would adapt their recipes based on resources available to them in their home communities. For instance, rather than using rice bran as a carbohydrate source, participants from parts of Africa pondered the feasibility of cassava flour or fish meal. Moreover, resource availability did not just refer to what items were physically present, but also what ingredients were culturally appropriate. For instance, a participant from Cameroon explained to me that in making animal feed using a fermentation process similar to bokashi, he could not justify using certain parts of fish or others would see him as "arrogant" for feeding animals what the people around him could not afford (Abo-Sido 2016, *unpublished*).

In theory, I could imagine substituting ingredients in bokashi based on available resources and soon after first learning about bokashi, I witnessed its malleability as an intern in a reforestation project with the Azuero Earth Project (AEP) on the Azuero Peninsula in Panama. Participants of the reforestation project at AEP were cattle ranchers, choosing to reforest fragments of their land to provide shade for cattle, diversify their livelihoods, and connect fragmented habitats. As a part of AEP's mission, the organization offered an organic reforestation program, using bokashi to fertilize transplanted tree seedlings. Bokashi was already being used vastly in Latin America (Rivera 2001), and I found a collection of different recipes developed across the region, that made use of available resources. Although the ingredients vary, the principle and process for creating the fertilizer remain the same. Thus, although the recipes I used to make bokashi in Japan and in Panama differed, there were critical parallels between the types of ingredients utilized and steps for making matured bokashi (Table 1).

While I valued the adaptability of bokashi, I continued to wonder about its efficacy and whether different starting ingredients would impact the quality and productivity of bokashi. I found that several studies have explored and confirmed the efficacy of bokashi as an organic soil amendment, contributing nutrients and fostering plant growth.

Ingredient	**Japan**	**Panama**	*Purpose*
Manure	Chicken	Cow	Rich source of nitrogen, as well as a source of phosphorus, potassium, calcium, magnesium, iron, manganese, zinc, copper, and boron
Rice husk	Smoked/Charcoal	Raw	Improves aeration, facilitates moisture absorption, provides medium for microbial activity; additionally, charcoaled rice husk increases soil pH
Microbes	Indigenous microorganisms (IMO)—also known as effective microorganisms (EM)	Baker's yeast (*Saccharomyces cerevisiae*)	Fermenters—break down materials
Carbohydrate/ bran	Rice bran	Concentrated animal feed (substitute for rice bran)	Supplies vitamins that promote fermentation; source of nutrients such as nitrogen, phosphorus, potassium, calcium, and magnesium
Soil	Soil from the farm or nearby forest	Soil collected from the riverbank	Filler for fertilizer
Other ingredients	*none*	Ash, calcium carbonate (agricultural lime), cane molasses	Ash and lime increase soil pH; molasses supplied substrate for yeast

Table 1: Comparison of ingredients used, and their purposes (Rivera 2001), when making bokashi according to the recipe I was taught in Japan and in Panama.

2.3 Effectiveness of bokashi

Published studies and accounts of the composition, use, and effectiveness of bokashi include approximately forty scientifically reviewed papers and a few books—at least those are what are available in English and/or Spanish and accessible digitally. Among these studies, several documented improved tomato, onion, pepper, coffee, and mango yield and growth upon the addition of bokashi (Álvarez-Solís et al. 2016; Aurora Gomez-Velasco et al. 2014; França et al. 2016; Lima et al. 2015; Peralta-Antonio et al. 2014). Taller tree heights upon amendment with bokashi have also been reported (Jaramillo-López, Ramírez, and Pérez-Salicrup 2015). Moreover, bokashi has increased soil microbial diversity in maize fields in Mexico (Bautista-Cruz et al. 2014) and in amended soils supporting *Alpina purpurata* (red ginger plant) (Saldaña et al. 2014). In addition, bokashi has catalyzed organic matter degradation by effectively contributing diverse microorganisms to soil (Aurora Gomez-Velasco et al. 2014; Boechat, Santos, and Accioly 2013).

Franca et al. (2016) found that varying the ratio of bokashi ingredients—perhaps in parallel with the amount of available resources—still produces productive fertilizers, but that it was essential that manure be present to significantly improve tomato plant growth. Lima et al. (2015) examined whether different types of manure—chicken and cattle—would affect the properties of bokashi, and subsequently, the growth of tomatoes. The authors concluded that while different types of bokashi may have variable effects on soil fertility and plant growth, differences in soil types can generate even greater variation; therefore, soil characteristics must be considered when assessing which amendments would be most productive. Moreover, the authors deduced that poultry litter—the flooring and droppings combined in chicken coops—may outperform cattle manure because chicken manure exhibits faster decomposition rates of organic matter (Lima et al. 2015). This observation has also been made by Boechat et al. (2013) while examining the effects of different organic waste materials on bokashi function. The study concluded that bokashi catalyzed organic matter degradation—with some variability between waste sources—given the escalation in percent nitrogen availability within the first seven days of bokashi maturation. The quicker generation of available nitrogen in bokashi treated samples relative to waste incubated without bokashi may support the advantage of shorter maturation times for bokashi relative to compost, without compromising nutrient availability (Boechat, Santos, and Accioly 2013).

3. Project goals

3.1 Mechanistic understanding of bokashi

Though the benefits of various types of bokashi fertilizer on soil fertility and plant growth have been explored, few studies exist that comparatively examine the effects of different ingredients on bokashi function (Lima et al. 2015; Boechat, Santos, and Accioly 2013). Even fewer aim to analyze the biochemical transformations of bokashi from starting ingredients and the mechanisms underlying the fertilizer's function (Boechat, Santos, and Accioly 2013). Moreover, researchers have identified possible microbes present in bokashi, but the relative microbial community composition of bokashi throughout the maturation process has not been described. Understanding nutrient transformation in conjunction with microbial community composition in the maturation of bokashi could provide mechanistic insight not only into the decomposition processes within bokashi piles, but also the efficacy of bokashi in supplying plant nutrients. In effect, an understanding of these processes could inform methods for optimizing bokashi production from available waste resources, effectively contributing to the adaptability of the fertilizer to different contexts.

In combination with an analysis of nutrient and microbial composition of bokashi throughout the maturation process from raw materials to bokashi fertilizer, I will compare the growth of crops grown in soils amended with bokashi relative to those in soils amended with compost to draw connections between nutrient composition and nutrient bioaccessibility.

3.2 Bridging the gap

By using a reductionist approach to contribute to modern ecological knowledge—which tends to be broad but shallow—and understand the intricacies of a traditional agricultural practice, I aim to bridge the gap between knowledge systems to reconcile the advantages of both approaches and generate knowledge both deep and broad. Vandermeer and Perfecto (2013) argue that a widespread challenge to combining scientific theory and native knowledge is the high uncertainty in our understanding of complex ecosystems. By assessing analytically the properties of bokashi, I am contributing to an agroecological approach to research by meeting testimonials of bokashi efficacy and economic sustainability with an enhanced understanding of system interactions. In effect, with a mechanistic understanding of bokashi, I aim to pull discussions of sustainable agroecological techniques into Western science and agricultural systems in the USA, while maintaining that the source of these innovative techniques stem from traditional knowledge. Ultimately, this approach seeks to reinforce the value of local knowledge, while assessing implications for global adaptability and contributing to sustainable agroecology and food sovereignty (Figure 2).

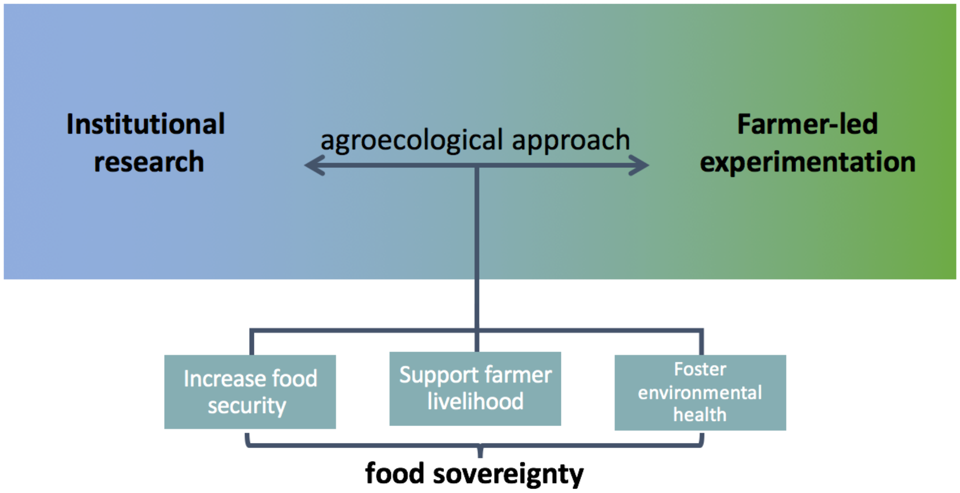

Figure 2: Concept map illustrating how a bidirectional exchange of knowledge in an interdependent science-approach could bridge the gap between scientific and local knowledge systems, effectively promoting food sovereignty.

Chapter 2

An analysis of the chemical transformation and composition of bokashi fertilizers

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1. Background

1.1 Plant nutrients

Among the nutrients essential for plant survival and growth, nitrogen (N) and phosphorus (P) are required in relatively large amounts and are critical to basic cellular function (White and Brown 2010). Both N and P make up DNA and RNA, the genetic material and carrier of said information, respectively, essential to all life. N and P are also integral components of adenosine triphosphate (ATP), which transfers much of cellular energy (Smil 1997; Blevins 1992). Proteins, including enzymes, are built from amino acids comprised fundamentally of N in amine functional groups. Plants require N for specific enzymes and pigments that power photosynthesis. Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase), regarded as the most abundant enzyme on Earth, is integral to the conversion of atmospheric carbon dioxide into energy-containing molecules, such as glucose. Rubisco is comprised of N and P, and studies have established that the Rubisco content and activity increase linearly with levels of leaf N (Cheng and Fuchigami 2000; Evans 1983; Amane Makino et al. 1992; A. Makino, Nakano, and Mae 1994; Amane Makino et al. 1997; Nakano, Makino, and Mae 1997). Each chlorophyll molecule, the major pigment that absorbs light to drive photosynthesis, is made up of four N atoms in the light-absorbing region (Morris et al. 2016).

Despite their natural elemental abundances, both N and P are often limiting in their availability and/or accessibility in agricultural systems. Natural cycling of these nutrients interconverts their forms, and these processes depend on microbial activity.

<u>1.1a: The nitrogen cycle</u>

The nitrogen cycle exemplifies the interconnectedness of microbial processes and the essentialness of microbes in driving global nutrient cycles (Figure 1). N comprises 78% of the atmosphere, but in the form of N_2, the nutrient is unusable by plants, and ultimately requires conversion into more bioavailable forms of nitrate (NO_3^-) and ammonium (NH_4^+)

(Smil 1997). Most N taken up by plants is recycled, initially sourced from the atmosphere but then converted into various forms by microbes and plants.

To tap into the dormant N_2 reservoir, microbes in the soil can—either independently or in symbiosis with legumes—undergo nitrogen fixation to convert atmospheric N_2 into the bioavailable, inorganic forms: ammonia (NH_3) or ammonium (NH_4^+), when dissolved. Ammonia can be further converted into nitrite (NO_2^-) and then nitrate (NO_3^-) by nitrification by microbes (Deenik 2006). In most soils, N is mainly in the form of solid organic N (from proteins and their monomer amino acids), and also remain inaccessible to plants until broken down, or mineralized, to ammonia/ammonium (Jones et al. 2005).

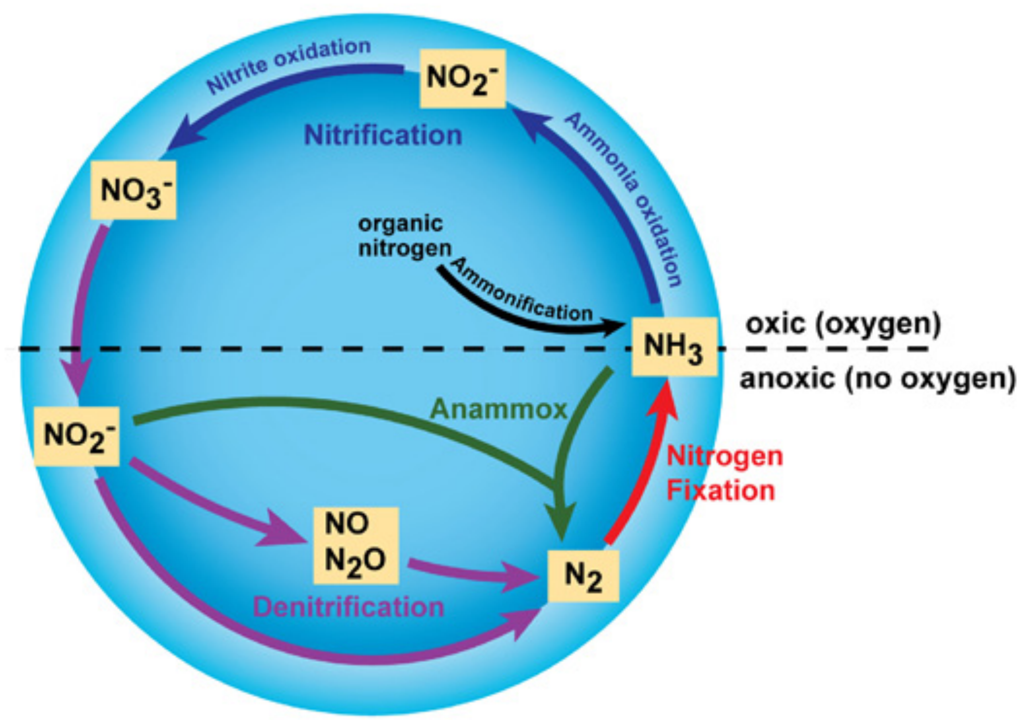

Figure 1: The nitrogen cycle from Bernhard, 2010 (Nature Education 2010).

N may be lost to the atmosphere by conversion to ammonia or nitrifying bacteria may convert ammonium to nitrate, which may be denitrified into N_2. Alternatively, the positively charged ammonium can sorb to negative clay surfaces and participate in cation exchange. Finally, the N may be immobilized by integration into soil microorganisms or plants, which eventually return organic nitrogen when decomposed (Deenik 2006).

1.1b: Phosphorus phytoavailability

Unlike the nitrogen cycle, the phosphorus cycle does not include a stable atmospheric reservoir (Filippelli 2008; Rodríguez and Fraga 1999). Microorganisms still play a critical role in P cycling as patterns of oxidation and reduction convert P from phosphine (oxidation state of -3) to phosphate (+5); but the microbial mechanisms that transform these chemical species are not completely understood. The most common forms of P taken up by plants are HPO_4^{2-} and $H_2PO_4^-$ (Rodríguez and Fraga 1999).

At the beginning of soil development, P is mostly in the form of calcium apatite minerals. Over time, the P minerals dissolve and either convert to organic phosphorus via utilization or become sorbed onto other minerals as inorganic P (Crews et al. 1995; Blevins 1992). The inorganic P is labile and can be desorbed by diffusion or uptake by microbes or plants. Alternatively, inorganic P that becomes surrounded by iron or aluminum oxides, or allophane (an alumino-silicate), is said to be occluded and is mostly unavailable to microbes and plants (Crews et al. 1995; Stewart and Tiessen 1987). The occluded form becomes more likely as the soil develops as more minerals dissolve. To release the P from soil components or convert them to available forms, several plants have formed symbiotic relationships with mycorrhizal fungi, exchanging sugars for extracted P. Other plants secrete acids that form complexes with soil metals or enzymes to release P or break down organic forms of the nutrient, respectively (Blevins 1992). Organic P can be recycled into inorganic forms or enter more dormant, decomposing, organic forms that do not participate nutrient cycling for extended periods of time (Crews et al. 1995).

Deficiencies in taking up essential plant nutrients—including N and P—will limit plant growth and, in effect, decrease crop production in agricultural settings. Given that plants mainly source these nutrients from soils, nutrient scarcities may be remedied by amending soils with fertilizers containing the required nutrients (White and Brown 2010). With the development of the Haber-Bosch process—an industrial synthesis of ammonia from atmospheric N (N_2) and hydrogen (H_2)—sourcing bioavailable nitrogen no longer depended solely on natural processes and could now be applied in abundance as a fertilizer. In effect, beginning in the 1960s, the Green Revolution marked an era of high-yield agriculture, and about half of the world population relies on this increase in yield for sustenance (Fowler et al. 2013; Sutton et al. 2011). It has become increasingly evident that this boom in agricultural yield is unsustainable as approximately half of applied fertilizers are lost to the environment, polluting bodies of water, causing eutrophication, emitting greenhouse gases, and presenting human health risks (Fowler et al. 2013; Sutton et al. 2011). Mediating these negative externalities is very costly, environmentally and economically (Fowler et al. 2013).

Nevertheless, supplying sufficient nutrients is critical to meeting yield demands. Amending soils using agroecological approaches may optimize natural nutrient cycling processes and effectively limit the negative consequences of high fertilizer inputs. Many small farmer agroecological techniques can meet high yields and foster biodiversity, effectively promoting dynamic nutrient cycling (Kremen 2015; De Schutter 2011). Global reports argue that within ten years, small farmers can double food production by utilizing these already existing agroecological methods (IAASTD 2009; De Schutter 2010; Altieri, Funes-Monzote, and Petersen 2012). Bokashi is an example of such a method, as it is an organic soil amendment that makes use of microbial processes to supply nutrients to agricultural systems.

1.2 Bokashi nutrients and biochemical transformation

1.2a: Nutrients

Various studies have illustrated the efficacy if bokashi as a fertilizer that improves plant growth (Álvarez-Solís et al. 2016; Lima et al. 2015; França et al. 2016; Bautista-Cruz et al. 2014; Aurora Gomez-Velasco et al. 2014; Peralta-Antonio et al. 2014; Jaramillo-López, Ramírez, and Pérez-Salicrup 2015; Boechat, Santos, and Accioly 2013) and several have investigated the amendment's nutrient content (Lima et al. 2015; Boechat, Santos, and Accioly 2013; Álvarez-Solís et al. 2016; Aurora Gomez-Velasco et al. 2014; Peralta-Antonio et al. 2014). Concentrations of N and P in bokashi appear highly variable, and may be due to the differences in ingredients or procedures in making bokashi across the studies (Table 1).

To date, no studies have compared the effects of variation in bokashi ingredients on nutrient composition, nor have any studies reported on the change in nutrient composition over the maturation of bokashi, or described the processes and pathways that lead to the development of the nutrient-rich fertilizer. To understand the processes that break down organic matter into bokashi, we must first consider the diversity of microbial metabolism.

Study	**N (mg/kg)**	**P**	**Bokashi composition**
(Álvarez-Solís et al. 2016)	Total N: 9500*	105.8* mg/kg	Ingredients (fermented for 30 days, aerobically): sheep, chicken, and cow manure; corn stalks, straw, green grass, green leaves, forest soil, yeast and molasses
(Lima et al. 2015)	Total N: 19800	n/a	Cow manure
	Total N: 31000	n/a	Mixture of plant remains, poultry litter, bone meal, and castor cake
	Total N: 28600	n/a	Chicken manure
(Boechat, Santos, and Accioly 2013)	NH_4^+: 263.40 NO_3^-: 171.08 Organic N: 3720	0.28 g/dm^3	Treated paper waste, lime (CaO)
	NH_4^+: 750.12 NO_3^-: 855.40 Organic N: 4490	4.04 g/dm^3	Waste from petrochemical complex industry
	NH_4^+: 8619.80 NO_3^-: 421.12 Organic N: 32630	9.49 g/dm^3	Treated urban sewage, lime
	NH_4^+: 6182.40 NO_3^-: 36.92 Organic N: 19200	15.00 g/dm^3	Treated organic waste from dairy industry: cheese, butter, and milk processing, lime
	NH_4^+: 460.60 NO_3^-: 881.72 Organic N: 19500	0.51 g/dm^3	Organic compost from byproduct of fruit processing
(Aurora Gomez-Velasco et al. 2014)	Total N: 15400	311 mg/kg	Sheep manure, sugarcane molasses, yeast
(Saldaña y Hernández et al. 2014)	NH_4^+: 63.27 NO_3^-: 627.43	10.16 mg/kg	Sheep manure, foliage, hay, soil, vegetal coal, yeast, molasses, lime, water; fermented for 20 days
(Peralta-Antonio et al. 2014)	Total N: 322	894 mg/kg	(composition not specified)

Table 1: Bokashi nitrogen and phosphorus content from various studies. Nitrogen values are total N, unless otherwise specified. Nutrient values are reported in mg/kg of dry bokashi. The ingredients used to make the bokashi differed between studies, so bokashi composition as noted per experiment is included—bokashi from the same studies contained the same ingredients but differed in the materials noted. “n/a” indicates no data available. “*” indicates that the study does not specify whether these nutrient values are per dry or wet sample.

1.2b: Metabolic activity

Different studies describe bokashi in variable ways and there is no single, comprehensive definition of the fertilizer. I deduce that this stems from both an incomplete understanding of the mechanisms governing bokashi maturation as well as the flexibility underlying bokashi production. Fundamentally, making bokashi involves generating an environment that promotes the breakdown of organic matter by a combination of aerobic respiration, anaerobic respiration, and fermentation (Boechat, Santos, and Accioly 2013; Álvarez-Solís et al. 2016). The promotion of aerobic processes distinguishes bokashi from traditional compost.

In respiration, the chemical potential energy from an organic molecule is converted to the chemical potential energy in ATP, in which energy can be conserved (Morris et al. 2016). Respiration involves the generation of energy by a series of redox (reduction and oxidation) reactions that create a proton motive force while transferring electrons from a donor to an acceptor (Kelly, Poole, and Hughes 2001). If the terminal electron acceptor is oxygen, the process is aerobic respiration; conversely, anaerobic respiration is categorized by any electron acceptor other than oxygen (Kelly, Poole, and Hughes 2001). Both types of respiration are far more energy efficient than fermentation, which is an anaerobic process characterized by the partial breakdown of organic compounds. In effect, the final products of fermentation—such as lactic acid and ethanol—are only partially oxidized and still contain significant potential chemical energy within their bonds (Morris et al. 2016).

Completely anaerobic methods of preparing bokashi (in closed containers) are often implemented in the composting of food waste like dairy and meat products that are typically excluded from aerobic composting processes, but this type of bokashi—often referred to as *bokashi compost*—is different than the agroecological technique of interest. To make bokashi fertilizer, the starting material must be covered in the initial stages to foster partially anaerobic conditions toward the pile centers, and aerobic regions layered outward (Boechat, Santos, and Accioly 2013). Moreover, the piles must be aerated daily, by mixing, to sustain aerobic conditions, prevent overheating, and conserve moisture.

Because of the diversity of microbial physiology, different microbes can occupy various niches within a system. Based on the environment and competition for resources, some microorganisms can thrive on different metabolic pathways, while others can only thrive with accessibility to specific biochemical pathways and therefore, resources. Among these potential pathways, energy yields increase with increasing reduction potential. The reduction potential describes the favorability of a chemical species to accept electrons—the more favorable, the higher the energy released upon this transfer of electrons (Figure 2). Oxygen is among the most energy-yielding electron acceptors; thus, many microbes will favorably undergo aerobic respiration in the presence of oxygen. In anaerobic environments, the absence of oxygen favors the reduction of other chemical species such as nitrate (NO_3^-), iron (Fe_3^+), sulfate (SO_4^{2-}), and carbon dioxide (CO_2) by anaerobic respiration (reference Vanja's notes). Alternatively, some microbes will preferably undergo fermentation even in the presence of oxygen, or ferment as a last resort when resources are not available for respiration.

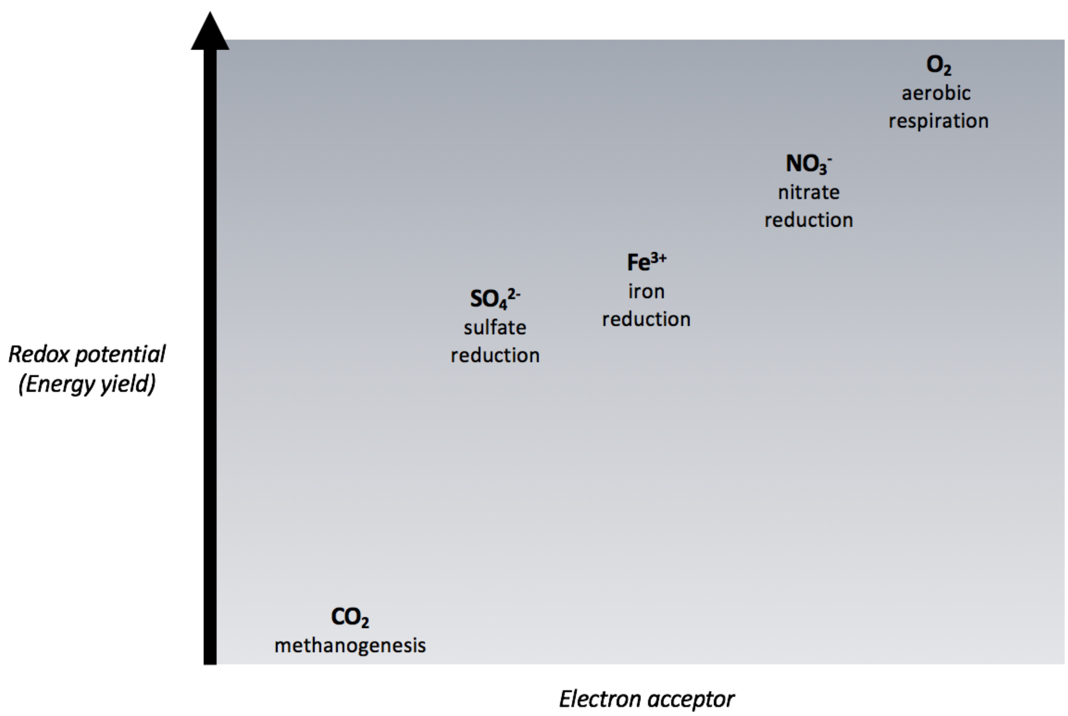

Figure 2: Redox tower of microbial metabolism. Terminal electron acceptors are plotted by increasing redox potentials

Facultative anaerobes will perform aerobic respiration if oxygen is present, but can switch to anaerobic processes in the absence of oxygen. *Escherichia coli*—a model organism in microbiology—is an example of such an organism and will essentially switch from aerobic respiration to anaerobic respiration to fermentation as conditions become anoxic and nutrient deficient (Unden 1998). Human muscle cells will preferably undergo aerobic respiration, but cannot perform anaerobic respiration, so will switch to fermentation in oxygen scarce conditions, such as during exercise (Morris et al. 2016). *Lactobacillus*, on the other hand, namely rely on fermentation for growth. The coexistence of microbes can be based on byproducts of one's metabolic processes powering the metabolism of another; yet, in the context of global nutrient cycles, the same fundamental processes can supply nutrients critical for plant life.

The regional variability in oxygen availability within bokashi promotes the coexistence of diverse microbes utilizing some combination of aerobic respiration, anaerobic respiration, and fermentation. In the hypoxic center, lactic acid fermentation partially breaks down organic waste material (Boechat, Santos, and Accioly 2013), and other anaerobic processes may be occurring. Sustaining high temperatures under covered bokashi piles drives accelerated oxidation of organic matter by microbes via a thermophilic phase (Álvarez-Solís et al. 2016). Furthermore, aerating piles is important for maintaining temperatures that can foster diverse microbes, occupying various physiological niches.

The marketed effective microorganisms (EM), developed by Dr. Teruo Higa, Ryukyus University, Okinawa, Japan, can be purchased and added to bokashi ingredients as an array of pre-selected microorganisms to supply diverse, beneficial microbes to maturing piles. The EM mix mainly contains lactic acid bacteria, yeasts, phototrophic bacteria, actinomycetes, and fungi (Ndona et al. 2012; Pei-Sheng and Hui-Lian 2002). Some studies have reported beneficial effects of EM-bokashi on soil nutrient content and plant growth (Xu 2001; Higa 1994), while others have found the effects of EM negligible to the non-specific addition of microbes from combining bokashi ingredients (Formowitz et al. 2007; Mayer et al. 2010). Alternatively to EM, baker's yeast, *Saccharomyces cerevisiae*, is also a common starter for accelerating fermentation in the making bokashi (Rivera 2001). Furthermore, a virtually free microbial starter commonly used are indigenous microorganisms (IMO), cultivated from local soils, and most frequently, soils from forest floors. The theory behind IMO use is that fostering the growth of diverse microbes from soils where organic matter degradation happens quickly will supply maturing bokashi with a mix of decomposing bacteria, similar to EM. Regardless of the microbial source, some microorganism starter culture is an important ingredient in bokashi production, to ensure accelerated decomposition of organic material into a productive fertilizer.

Other integral bokashi ingredients include a rich nitrogen source—often manure—and carbohydrates. Carbohydrates may be brans or flours, and rice hulls or other dry matter and they may serve not only as food for microbes, but also media for microbial growth and moisture control. According to Rivera (2001), concentrated animal feed can—and has been used in Panama—as a more affordable, nutrient-dense carbohydrate source in place of brans. Other studies have used crushed sugarcane (França et al. 2016), compost , or other organic wastes like paper or fruit-processing waste (Boechat, Santos, and Accioly 2013).

Prior to adding them to bokashi piles, rice husks may be smoked to generate a material analogous to biochar. In effect, smoked rice hulls—like biochar—can decrease soil acidity, add soil organic matter, supply P, enhance cation exchange capacity, and improve the flow of potassium and calcium, other essential plant nutrients (Masulili, Utomo, and Syechfani 2010). Alternatively, lime and/or ash may be added to bokashi to increase soil pH upon application (Rivera 2001; Saldaña et al. 2014; Boechat, Santos, and Accioly 2013).

1.3 Experimental goals and hypotheses

Few studies analyze the effects of different ingredients on bokashi nutrient content (Boechat, Santos, and Accioly 2013; Lima et al. 2015) and no comprehensive account of the biochemical transformation during bokashi production—one that describes the nutrient composition over time—from its starting materials to a productive fertilizer has been fully reported. The goal of this study is twofold: (1) to measure the nitrate, ammonium, and phosphate composition of different types of bokashi, and (2) to follow the generation (and consumption) of these nutrients over the maturation of bokashi from its starting ingredients.

To address these goals, I made three types of bokashi with variable starting ingredients, substituting raw rice hulls for smoked rice hulls or indigenous microorganisms (IMOs) for yeast, and measured their nutrient content over 12 days: from combining ingredients to bokashi maturation. Any variation in the nutrient composition or maturation of bokashi piles across different treatments may inform ideal ingredients for the most nutrient-rich bokashi types.

2. Methods

2.1 Making bokashi

While the ingredients required to make bokashi are flexible, certain components and processes must be preserved to make a successful fertilizer. Bokashi must be kept covered for much of the process to limit oxygenation and effectively drive fermentation and anaerobic respiration, as well as to prevent excess moisture. Excess water may promote increasingly anaerobic environments, disrupting the aerobic-anaerobic balance of regions in maturing bokashi piles. This would occur as water fills previously air-filled pores, disrupts oxygen diffusion, and increases nutrient mobility and subsequent spikes in metabolic activity following drying (Tiedje et al. 1984). Consequently, over-moistened piles tend to release unpleasant odors and aggregate into chunks. Furthermore, maturing piles must be aerated regularly to prevent over-heating, and consequent selection of only thermophilic microbes.

I made three types of bokashi with variable starting ingredients, substituting raw rice hulls for smoked rice hulls or indigenous microorganisms (IMOs) for yeast. I designed the recipes and process that I used to make bokashi for this study based on what I had learned by making bokashi in Japan and Panama, as well through literary research (Rivera 2001). I adapted the procedures I learned based on the types and amounts of resources available to me, as well as the climate and conditions of the space in which I made the bokashi piles.

Each type of bokashi included approximately 2 gallons of cow manure, 3 gallons of soil, and 1 gallon of corn flour. All measurements were made using a marked 5-gallon bucket. I collected fresh manure from a pasture at the Natick Community Organic Farms (Natick, MA), a certified organic farm approximately 3 miles from Wellesley College. I sieved the manure prior to adding it to bokashi piles to break the material down into smaller pieces and effectively promote greater homogenization. The soil was “Coast of Maine” organic topsoil, consisting of loam, compost, and peat. The corn flour was Bob’s Red Mill organic whole grain, stone ground corn flour.

In addition to the base ingredients, the first set of piles received 5 g *Saccharomyces cerevisiae* yeast and 50 mL molasses and 1 gallon of either raw rice hulls or charcoaled/smoked rice hulls. In the second set of piles, both types of bokashi were made with 1 gallon of raw rice hulls and while one treatment was made with the yeast (and molasses) starter microbes, the second treatment was made using IMOs cultivated from soils in terrariums[5] sitting in the Wellesley College temporary greenhouse as well as 50 mL of molasses. The yeast used in both sets of experimental bokashi was Fleischmann's® Active Dry yeast and the molasses: Crosby's Fancy Molasses. Procedures for cultivating the MOs and smoking the hulls are described below, in sections 2.1a and 2.1b.

I made the bokashi piles in two rounds of experiments, separated by time. The first experiment involved making bokashi with either raw rice hulls (BR) or charcoaled rice hulls (BC). The second experiment involved making bokashi with either yeast (BY) or IMOs (BI). I made four replicates of each pile and all piles were made inside the same greenhouse, maintained at a temperature of approximately 22-25˚C. BC and BR were made at the same time, and BY and BI were made together after. All the ingredients were placed on a black plastic tarp on tables in the greenhouse and water was added to moisten the piles to approximately 60%. To gage this estimate, I used the traditional "ball test" or "prueba de pulga" used in both Japan and Panama to determine whether the piles were sufficiently moist. The test involves taking a handful of the bokashi ingredients and squeezing them tightly into a ball. If water is dripping out of the fist, the mixture is too wet and more ingredients must be added to soak up excess moisture. If the ball does not stick together, but instead crumbles upon release of the fist, then more water must be added (Rivera 2001). This amounted to approximately 1.5 L of water per pile. The piles were covered with tarp for the first seven days and mixed twice daily for the first three days, then once daily for the following 8 days, until fully dry on day 12.

BR and BY—although made at different time points and serving as variables in different sub-experiments—were made using identical ingredients—except manure

[5] Terrarium soils were used because the snow on the ground created a barrier to collecting forest soils for cultivating IMOs. Nonetheless, the terrarium soils had previously had plants and spent considerable time in the greenhouses and, thus, were predicted to contain a diverse supply of microbes, as a forest would.

collected at different time—and, thus, effectively served as a control between experiments. In other words, both BR and BY were made the same way, but at different times, and the qualities of their matured products may provide insight into the consistencies and variation in bokashi as it is repeatedly made.

2.1a: Smoking rice hulls

The procedure for smoking rice hulls for BC was adapted from experience and online research (Agribusiness How It Works n.d.). I obtained a metal pipe sheet approximately 3.5 feet by 1.5 feet in dimension and, using a mallet and nail, punched holes approximately 2 inches along the sheet. I then rolled and secured the metal pipe.

In a fire pit, the metal smoker was placed above a small fire, and then the base of the smoker was surrounded with raw rice hulls (Figure 2). The hulls were smoked for approximately 4 hours, then removed and watered to extinguish any embers. Successfully smoked charcoal is black; white regions of smoking rice hulls signified ash, which was not the intended product but was also produced alongside the charcoal.

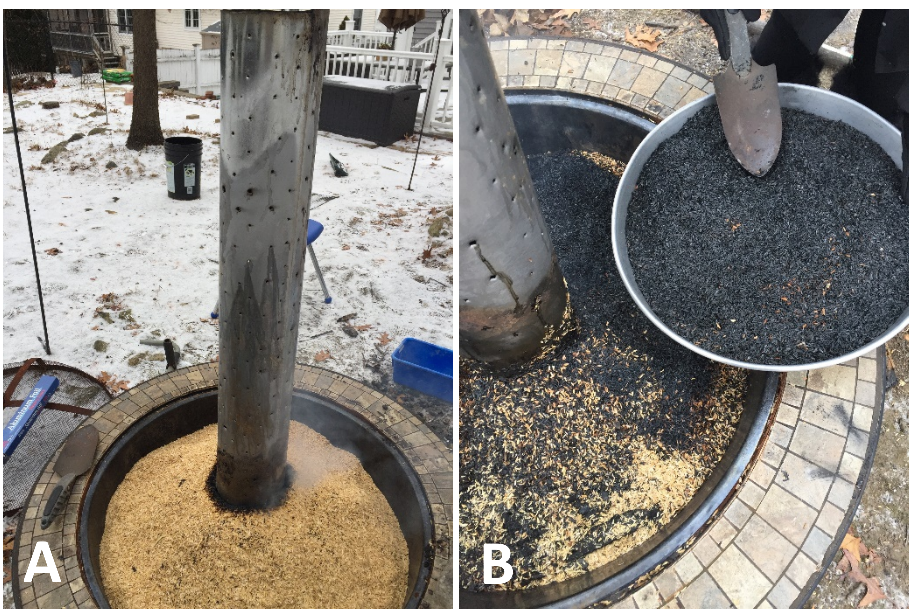

Figure 3: (A) Rice husk smoker and (B) smoked rice husks.

2.1b: Culturing IMOs

The procedure for culturing IMOs was adapted from experience, material from the Asian Rural Institute, and online research (Park and DuPonte 2008). I cooked white rice using only water, rolled the rice into many balls, covered the balls in terrarium soil in a bag, and hung the bag up in the greenhouse. Three days later, I scraped visible growth—filamentous white, milky white, yellow, blue-green microbes—from the rice balls (Figure 4) and placed them in a glass jar with dark brown sugar at an approximate ratio of 1 part brown sugar to 3 parts rice IMOs. I covered and shook the jar. Three days later, I added the mixture to a pile of approximately 1 gallon each of soil and corn flour. I added water to the pile using the ball test to bring it to about 60% moisture. I covered and mixed the pile each day for about a week, at which point, I added 2 handfuls of the IMOs to the BI piles.

Figure 4: Culturing IMOs. Bag containing local soil and cooked rice. Circles surround regions of harvested microbial growth.

2.2 Sample collection and analyses

I recorded the temperature of the center of the piles each day prior to mixing. I took a heat map of the piles using a Seek Thermal Compact infrared camera (Seek Thermal Inc., Santa Barbara, CA) to visualize the heat distribution of the piles as a proxy for microbial activity.

Every three days, from the first day of combining the ingredients (Day 0) to the final day of maturation (Day 12—at which point the bokashi was dry and ready for use), I collected 4 handfuls of samples from different parts of the piles using a gloved hand. I further homogenized each of the samples by mixing and then splitting each representative sample into two bags. Half of each sample was placed into a drying oven at 45˚C for approximately 4 days and the other half of each sample was frozen. Frozen samples were used for DNA extraction and metagenomic profiling (Appendix A).

Dried samples were ground for 2 minutes via a mixer mill, and the powdered samples were used to make extracts for colorimetric determination of ammonium, nitrate, and phosphate by an Astoria-Pacific Discrete Analyzer (Astoria-Pacific, Inc., Clackamas, OR, USA). The ammonium- and nitrate-test extracts were made by combining approximately 7 grams of sample and 40 milliliters of 2 M KCl to a 50-mL centrifuge tube, shaking the samples for an hour, allowing samples to settle for an hour, and then filtering the samples through a 2.5 μm (Whatman grade 42) filter paper to collect the extract. Concentrations of ammonium and nitrate (following reduction by cadmium) were determined based on absorption associated with indophenol blue (660 nm) and Griess azo dye (540 nm), respectively. Phosphate test extracts were made using the "Bray" method by combining 3 grams of sample with 25 mL of 0.1% NH_4F and 0.2% HCl, shaking for 2-5 minutes, settling for an hour, and then filtering through a 2.5 μm (Whatman grade 42) to collect the extract. Phosphate concentration was determined based on the absorption associated with molybdenum blue (660 nm). To measure pH, I prepared samples by combining 1 g ground sample and 1mL deionized water and measured the pH of the solution using a pH meter.

Analysis of Variance (ANOVA) and repeated measures ANOVA were used to examine variation in responses variables based on bokashi treatment type, time since the start of bokashi development, and their interaction. The Tukey Test was used to look for pair-wise differences among bokashi types on the final day of maturation (day 12). Analyses were performed using R (R Core Team 2014), with the 'ez' package (Lawrence 2016) used for repeated measures ANOVA.

3. Results

3.1 Temperature patterns

The temperatures of the bokashi piles followed the general trend of increasing before peaking at 40-50˚C between days 5 and 7 and then dropping in temperature following pile uncovering on day 7 (Figure 5). Thermal imaging of the piles illustrates a heat gradient in which the piles appear hottest at the center and cooler toward the outer regions (Figure 6). The center of the pile, on day 6, was approximately 35˚C and the outer regions from the center were at lower temperatures, the outer-most layer being approximately 23˚C.

3.2 Nitrogen: ammonium and nitrate

Ammonium levels (milligrams nitrogen from ammonium per kilogram dry mass of sample, or parts per million) exhibited variable trends over time by type (Figure 7), but in most cases illustrated a trend of increasing ammonium levels over increasing maturation time. Types BY and BI—the treatments from experiment 2—followed this trend consistently, while BC and BR—the treatments from experiment 1—exhibited fluctuations in ammonium composition over the 12 days. Ammonium levels were still generally highest for BC piles on day 12. BR piles appeared to peak in ammonium concentration on day 3, before decreasing steadily. Moreover, BR and BY bokashi types were identical in ingredients, but varied in the times at which they were made and the manure batch, and they exhibited different trends in ammonium levels over time: BY increasing steadily, while BR increased initially before declining.

Nitrate (milligrams nitrogen from nitrate per kilogram dry mass of sample, or parts per million) levels remained constant over time across all bokashi types (Figure 8).
A comparison of the trends in nitrate and ammonium levels over time illustrates no relationship between the concentrations of the two inorganic forms of nitrogen in any of the bokashi types (Figure 9).

There was a significant interaction between treatment and time for ammonium, but not nitrate concentrations. There was no significant difference between treatment type and final ammonium and nitrate concentrations (on day 12) (Appendix B).

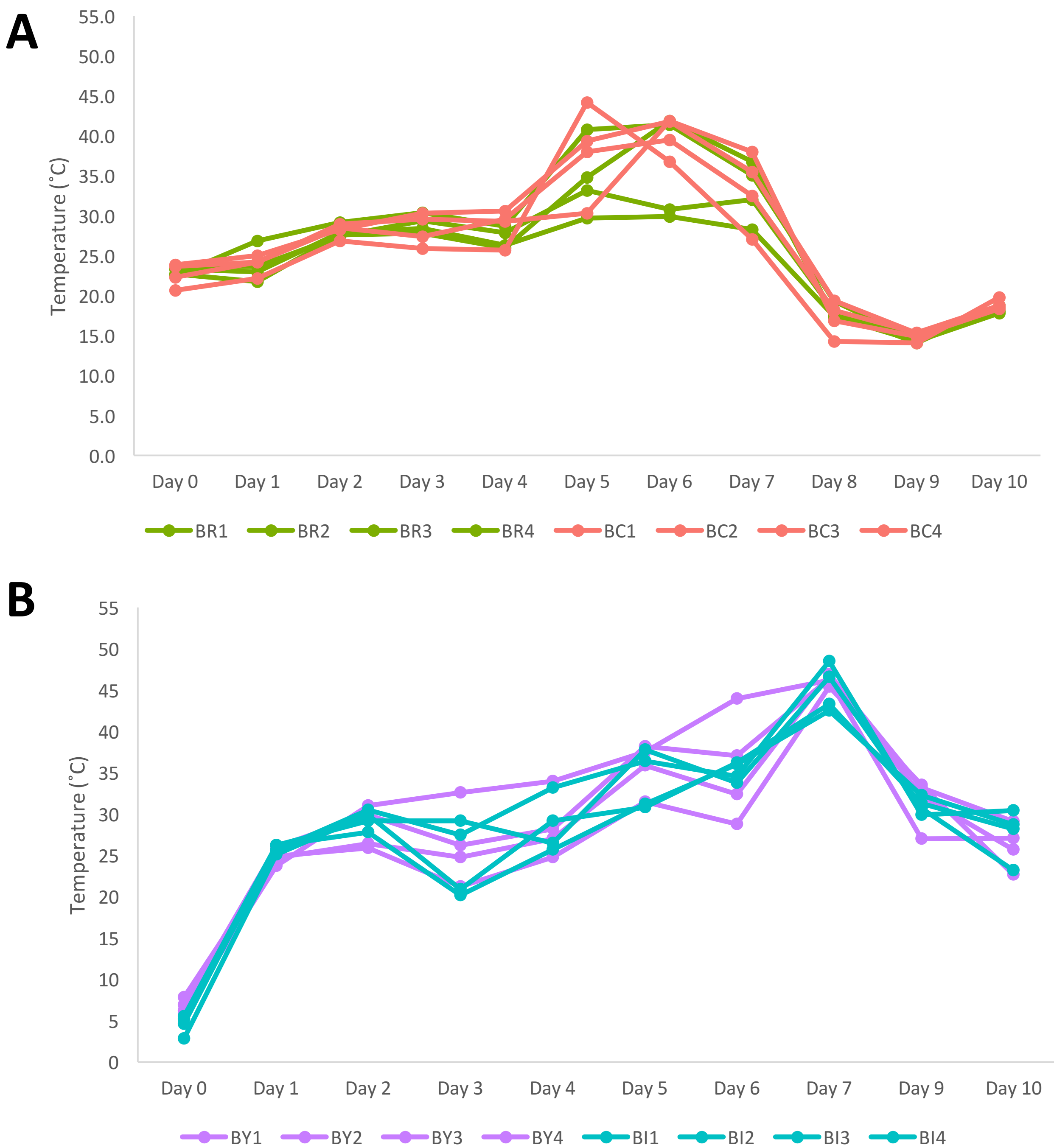

Figure 5: Temperatures of bokashi piles throughout maturation. The temperatures of each bokashi treatment and all four replicates were measured starting from the combination of ingredients on Day 0 through maturation to Day 10. Temperatures for the maturation of (A) Experiment 1 bokashi piles, BR and BC, were taken daily and the greenhouse ambient temperatures were approximately 22-25˚C, except on days 8 and 9 when the ambient temperatures fell to 17.5 and 15˚C, respectively. Temperatures during the maturation of (B) Experiment 2 bokashi piles, BI and BY, were taken daily. On Day 0, the greenhouse T dropped to freezing; on all other days, it was in the range of approximately 24-33˚C.

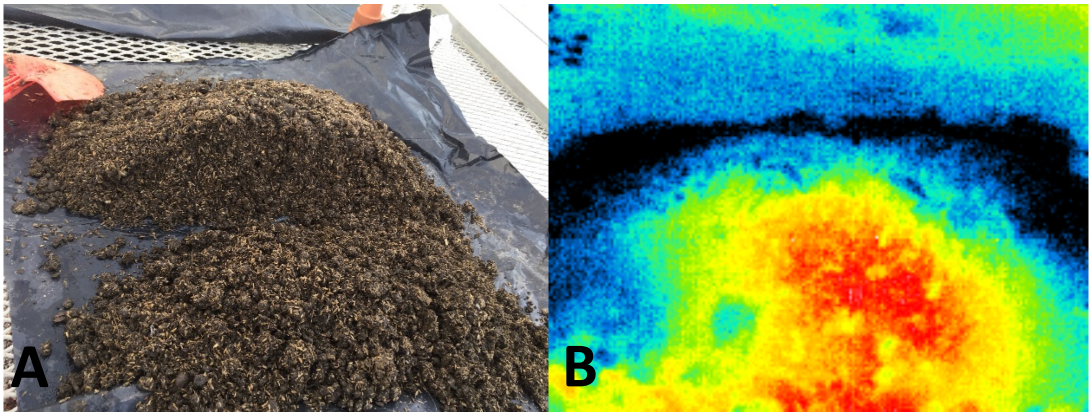

Figure 6: Thermal image of divided bokashi pile. (A) Photograph of BI bokashi pile (Experiment 2), on day 6, split in half and (B) the corresponding thermal image taken using an infrared camera. The temperature range from blue to red is approximately 23-35˚C.

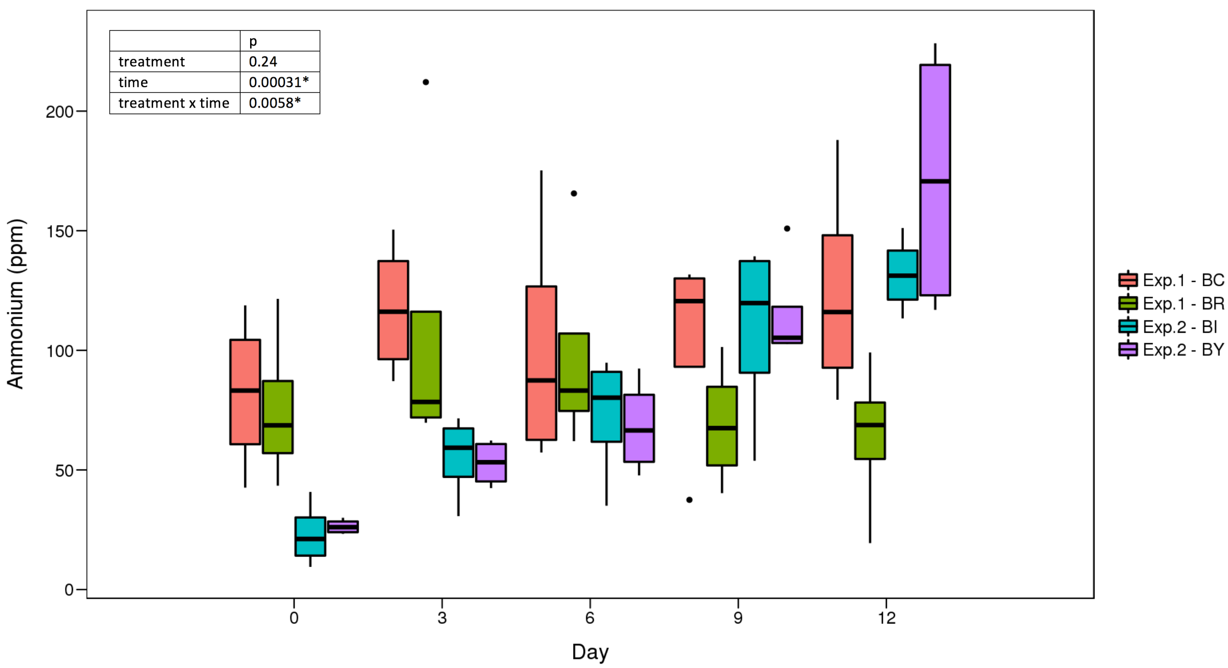

Figure 7: Bokashi ammonium concentrations over maturation time. The ammonium concentrations of each type of bokashi were measured across the development process. The table presents ANOVA results; asterisks (*) denote significance ($p < 0.05$).

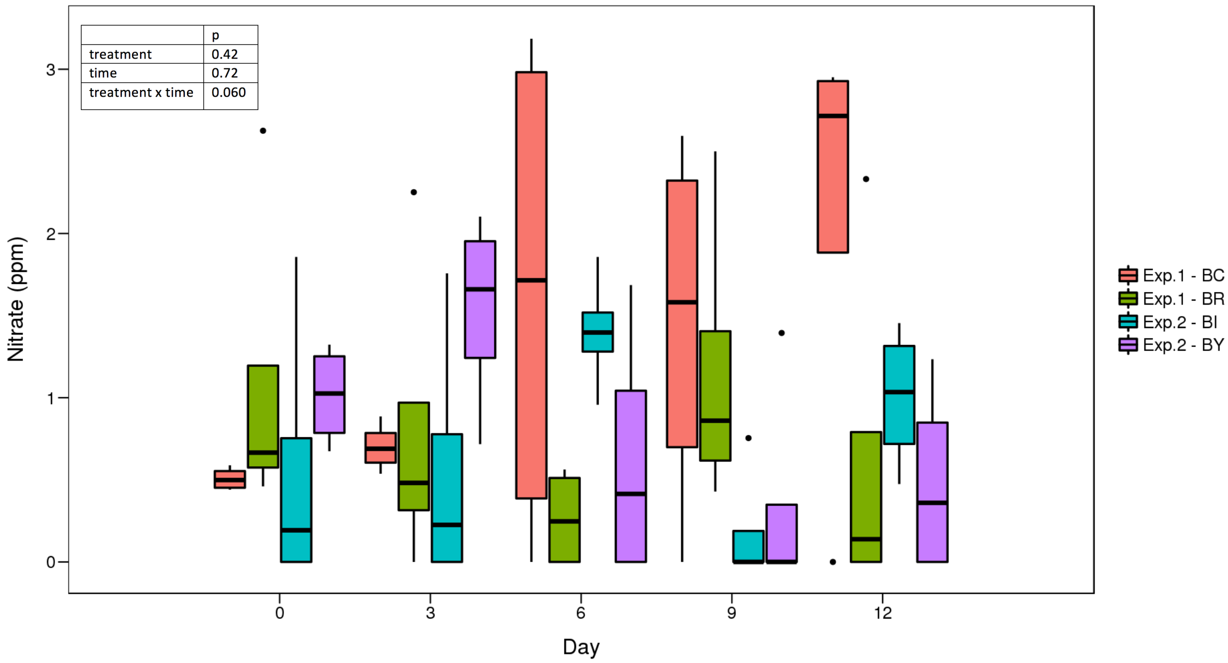

Figure 8: Bokashi nitrate concentrations over maturation time. The nitrate concentrations of each type of bokashi were measured across the development process. The table presents ANOVA results; asterisks (*) denote significance ($p < 0.05$).

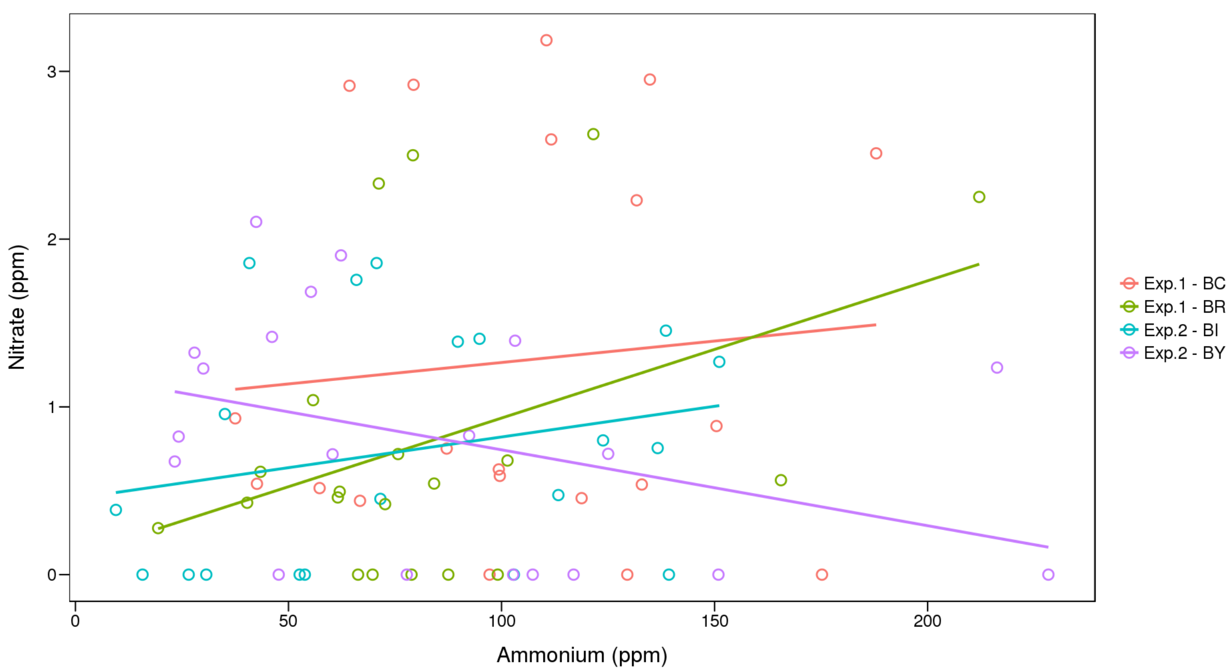

Figure 9: Bokashi nitrate and ammonium concentration. Lines are linear regressions.

3.3 Phosphorus

Phosphate levels (milligrams phosphorus in phosphate per kilogram dry mass, or parts per million) across all bokashi types exhibited a general decrease over time (Figure 10). There was a significant interaction between treatment and time for and phosphate concentrations for BI and BY were initially higher—between days 0 to 3—than those of BR and BC. There was no significant difference between the final phosphate concentrations of the different bokashi types (Appendix B).

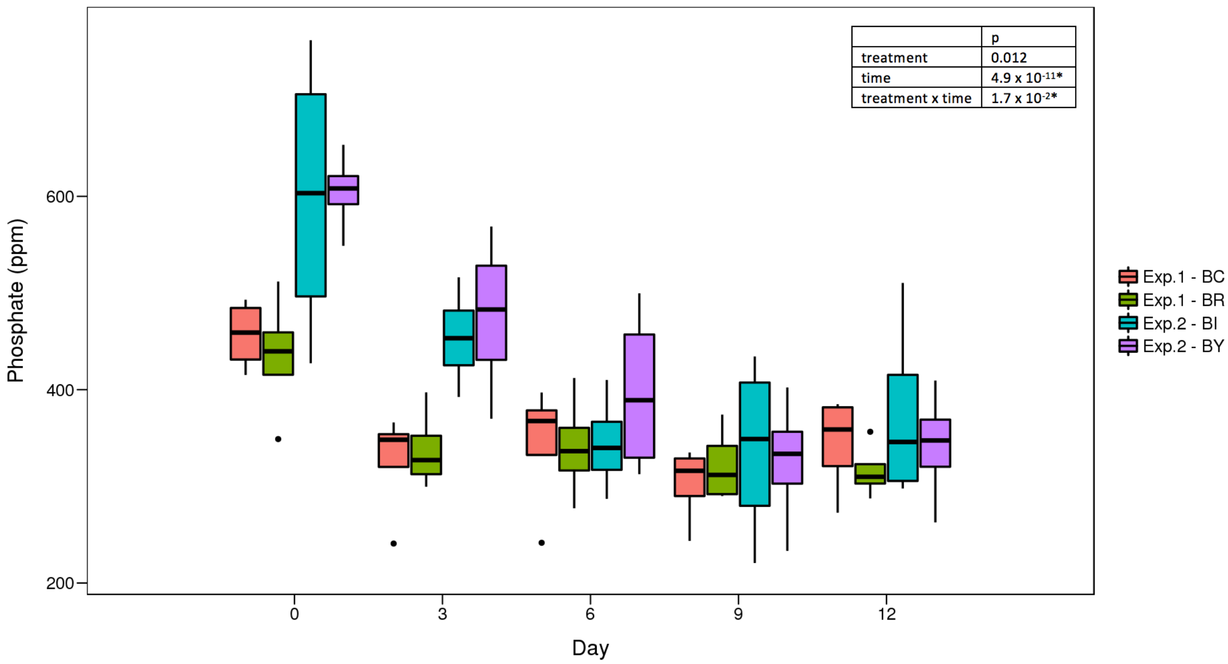

Figure 10: Bokashi phosphate concentrations over maturation time. The phosphate concentrations of each type of bokashi were measured across the development process. The table presents ANOVA results; asterisks (*) denote significance ($p < 0.05$).

3.4 pH

pH levels for all bokashi types exhibited an increase between days 0 and 3 and then remained relatively consistent through maturation (Figure 11). When the ingredients were first combined, the mean pH of all bokashi types was approximately 5.78, and by the end of maturation (day 12), the mean pH of all types was approximately 6.04. However, the BR type showed much less variation through time, and the pH of the BI type tended to decrease following its initial peak on day 3.

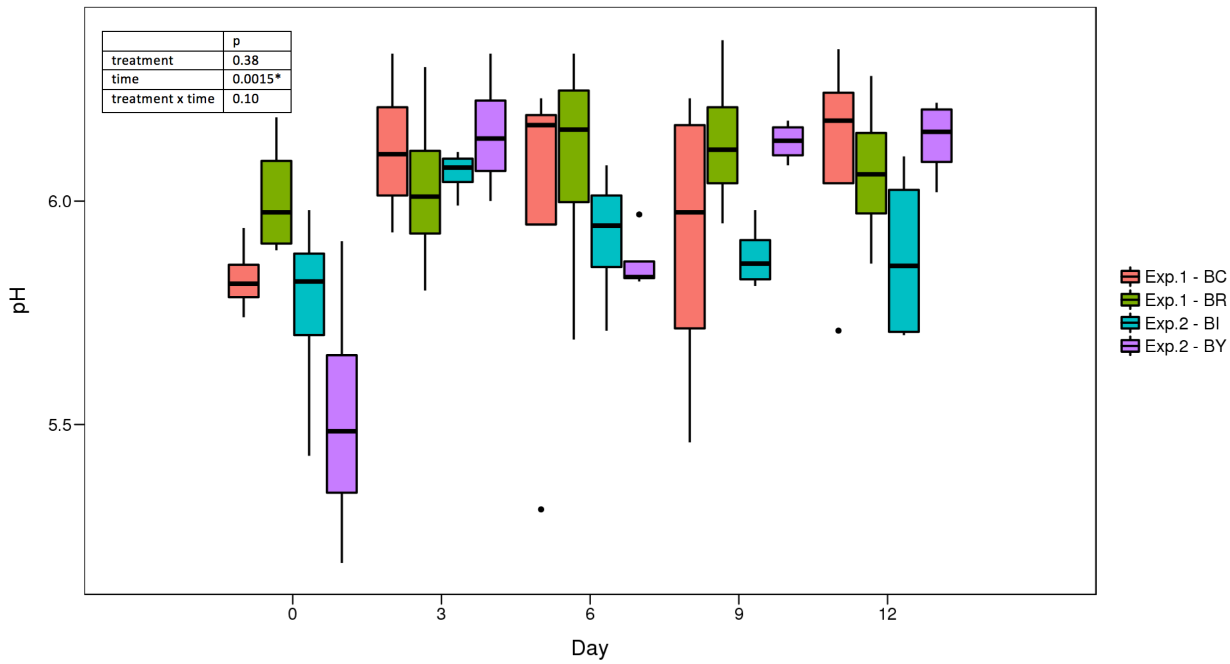

Figure 11: Bokashi pH over maturation time. The pH of each type of bokashi was measured across the development process. The table presents ANOVA results; asterisks (*) denote significance ($p < 0.05$).

The nutrient composition of bokashi in comparison to that of the soil and manure that went into the bokashi are summarized in Table 2.

	Ammonium (NH_4^+) (mg/kg)	Nitrate (NO_3^-) (mg/kg)	Phosphate (PO_4^{3-}) (mg/kg)
Bokashi	120	0.76	338
Soil	7.43	0.34	98.0
Manure	Exp.1: 90.4 Exp.2: 133	Exp.1: 3.07 Exp.2: 3.63	Exp.1: 680 Exp.2: 1810

Table 2: Median values of nutrient concentrations of bokashi (day 12), soil, and manure from both experiments (collected from the farm at different times).

4. Discussion

The goals of this study were to examine the nutrient content of bokashi made from variable starting ingredients and to analyze the variation in nutrient composition over the maturation of bokashi from starting ingredients to a fertilizer. There were no significant differences in the concentrations of ammonium, nitrate, and phosphorus at full maturation of the different types of bokashi. During the development process, ammonium levels increased, nitrate remained the same, and phosphate decreased.

4.1 Nitrogen: ammonium and nitrate

Naturally, ammonium is produced either by nitrogen fixation or mineralization/ammonification (Figure 1). In the bokashi systems, nitrogen fixation would occur by free-living heterotrophs, coupling oxidation of organic or inorganic compounds the conversion of atmospheric N_2 into ammonia. Nitrogen fixation requires the enzyme nitrogenase, which is inhibited by the presence of oxygen (Wagner 2011). It is possible that microbes may have been fixing nitrogen in the anaerobic microclimate at the center of bokashi piles, contributing to the increasing concentration of ammonium over bokashi development. However, frequent aeration would suggest that nitrogen fixation would be less of an ammonium contributor than mineralization, the conversion of organic N—such as that from proteins and amino acids—into ammonium.

High temperatures stimulate mineralization, and maximum mineralization occurs when soil temperatures reach 30-35˚C (Deenik 2006). At maximum temperatures on days 5-7, most bokashi piles reached and sustained temperatures over 30˚C, before declining to ambient temperatures upon uncovering the piles on day 7 (Figure 5). Moreover, the piles were frequently aerated, and the presence of oxygen promotes N mineralization (Deenik 2006). N mineralization is also promoted by the presence of rich carbon sources, given that decomposers generate energy from the breakdown of organic materials (Deenik 2006) The increase in N mineralization over the first ten days of bokashi development has been suggested by Boechat et al. (2013), and the overall increase in ammonium over the maturation of bokashi may support this.

While BI and BY bokashi types exhibited consistent increases in ammonium over time, BC and BR underwent more variable trends in ammonium concentrations over time

(Figure 7). BC and BR were made at the same time with the same manure, whereas BI and BY were made at the same time—and at a time different than the first experiment—and, thus, contained different manure (although from the same source as the first experiment). Because BR and BY were identical in recipe and only differed in the time at which the manure was collected, they effectively served as controls for study of potential variation in bokashi qualities just by variability in identical ingredient qualities. The manure used in the second experiment contained higher levels of ammonium, nitrate, and phosphate than the manure used in the first experiment (Table 2). This variation in manure nutrient levels could have been due to random variation in cattle manure quality, or it may have been the result of initial freezing and thawing of the manure used in experiment 1, whereas the manure in experiment 2 was used soon after harvest.

Nevertheless, I would have predicted that regardless of starting ammonium concentrations, all bokashi piles would have experienced similar trends in ammonium levels over time. Given that the trends differed between experiments, perhaps there are manure qualities beyond initial ammonium concentration that have affected the maturation of bokashi.

Nitrate levels remained relatively consistent over time for all bokashi types, with some samples potentially exhibiting concentrations below the limit of detection for the discrete analyzer (i.e. not different from 0 ppm standards), and no sample exceeded 3 ppm (Figure 8). Because there was no relationship between ammonium and nitrate concentrations in bokashi piles (Figure 9), the conversion of ammonium to nitrate by nitrification could not have been limiting nitrate speciation. Rather, nitrate consumption processes may have been happening at rates nearly equal to nitrate production, effectively resulting in no significant changes in nitrate concentrations over time in any of the bokashi types. Soil nitrate sinks include nitrate reduction by denitrification or dissimilatory nitrate reduction to ammonium (DNRA). Both denitrification and DNRA occur namely under anaerobic conditions (Giles et al. 2012), and ammonia fermentation is a form of DNRA performed by denitrifying fungi (Zhou et al. 2002). In particular, the genes involved in denitrification will be suppressed in the presence of oxygen, as facultative anaerobes will preferably undergo aerobic respiration in the presence of oxygen (Giles et al. 2012; Berks et al. 1995; Hernandez and Rowe 1987). These nitrate conversion processes may have occurred in the anaerobic center of bokashi piles.

4.2 Phosphate

Overall, phosphate levels for all types of bokashi decreased over time (Figure 10). During the initial development stages—between days 0 and 3—the phosphate levels of BI and BY were higher than those of BC and BR. The manure used for making BI and BY (experiment 2) contained nearly three times more phosphate than the manure used for making BC and BR (experiment 1). While the manure was sourced from the same source, the manure for experiment 1 was frozen prior to use, whereas the manure for experiment 2 was used soon after collection and the freezing and thawing process may have altered the nutrient chemistry of the manure. Regardless of these varying initial concentrations, there was no significant difference in phosphate levels of the different types of bokashi at the end of maturation (day 12).

The phosphate levels of the bokashi made in this study are generally higher than those reported in previous studies (Table 1; Table 2). The discrete analyzer measured extracted orthophosphate, whereas the studies either reported on total phosphorus or did not specifically indicate the forms of phosphorus measured, so differences in extraction and measurement procedures make direct comparisons between studies challenging (Wuenscher et al. 2015). For instance, Stewart and Tiessen (1987) address the concern of variable methods influencing reported phosphate levels by noting that some forms of organic P extraction may actually produce orthophosphate by hydrolysis, causing higher levels of phosphate to be detected than what would have been truly present.

Nevertheless, these values—like previous studies—illustrate the higher phosphorus levels in bokashi relative to soils (Table 2) (Aurora Gomez-Velasco et al. 2014; Peralta-Antonio et al. 2014; Boechat, Santos, and Accioly 2013; Álvarez-Solís et al. 2016). Boechat et al. (2013), reasons that elevated phosphate amounts in bokashi treated soils is due to the prevalence of acid tolerant microbes in bokashi that flourish and participate in the breakdown of organic material (Shen et al. 2011; Boechat, Santos, and Accioly 2013).

Inorganic P is labile and may be taken up by microbes or be occluded by iron or aluminum oxides. The decrease in phosphate in bokashi may be attributed to use by microorganisms for biomass (as in the phospholipid bilayer of every cell) or metabolic process (like for generating ATP). Organic P cycles rapidly as predators and decomposers take up biomass P, and other microbes make use of secreted or lysed organic P reservoirs (Stewart and Tiessen 1987). On the other hand, organic P may decompose slowly, remaining in stable organic forms for long periods of time, or be occluded by interactions with mineral. P occlusion is not permanent, and occluded P may be cycled into organic P by organisms that may release signals to dissolve the P (Crews et al. 1995). Ultimately, organic P utilization by microbes and P occlusion present potential pathways that lead to decreases in phosphate concentrations over the maturation of bokashi.

5. Conclusion

The gradient in oxygen availability and temperature from the center to outer regions of bokashi piles generates anaerobic and aerobic microenvironments where diverse microbes may perform variable metabolic processes. This variability in environments, resources, and occupied niches likely fosters coexistence in microbial communities.

While N mineralization likely contributed to increasing ammonium concentrations in maturing bokashi piles, free-living heterotrophs may have been behaving anaerobically in fixing N_2 to ammonia, potentially contributing to increased ammonium concentrations over time (particularly in the second experiment). Moreover, the abundance of ammonium and absence of a correlation in ammonium and nitrate concentrations suggest that ammonium did not limit nitrate production. Rather, while nitrification may have produced nitrate from ammonium, nitrate reduction by anaerobic respiration and denitrification served as nitrate sinks. These processes taken together may explain the consistent nitrate levels over bokashi maturation; however, it is difficult to know the overall magnitude of nitrates sources and sinks. Meanwhile, phosphate levels decreased over time as the nutrient likely cycled between labile and stabile forms, as well as organic and occluded reservoirs.

The pH of the all types of bokashi was initially relatively low, at a mean of 5.78 on day 0. Between days 0 and 3, the pH quickly increased and remained relatively consistent through maturation to day 12, when the mean pH of all types was approximately 6.04. This plateau in pH values coupled with the plethora of metabolic activity promoting nutrient cycling may be the result of buffering systems within the soil making up the bokashi as well as the coexistence of microbes on diverse substrates.

Measurements of the nutrient composition, temperature changes, and pH of the bokashi piles over maturation time have provided insight into the potential processes involved in the conversion of bokashi from starting materials to an ammonium- and phosphate-rich fertilizer. Nevertheless, the complexities of the system require further probing of nutrient content and microbial communities (Appendix A) to trace patterns of nutrient cycling and identify the critical organisms and processes involved in bokashi production.

Ultimately, the nutrient content of bokashi did not vary significantly when raw versus smoked rice hulls were utilized, or when yeast or IMOs were added to piles. Rather, variations in trends in ammonium and phosphate concentrations differed between experiments. Because the experiments were conducted at different times, the manure used were collected and stored at different times. Regardless of this variation, final ammonium and phosphate levels in all types of bokashi did not differ significantly and were high. Dynamic nutrient cycling in bokashi produces a nutrient-rich amendment and neither the identity of the starting ingredients nor the added microbial community significantly impacts the final nutrient composition of bokashi.

Chapter 3

Effects of different types of bokashi on the growth of cucumber and kale plants

1. Introduction

1.1 Bokashi improves plant growth

Diverse motivations for exploring the use of bokashi as an alternative fertilizer include the local availability of bokashi ingredients, the search for a more productive organic fertilizer, the high costs of manufactured fertilizers, the negative effects of chemical fertilizers on environmental and human health, and, ultimately, its potential to improve farmer livelihoods (Álvarez-Solís et al. 2016; Lima et al. 2015; França et al. 2016). An advantageous characteristic of bokashi is that it can be made with different ingredients, so long as some source of nitrogen, carbohydrate, and active microbes are included and processes foster partially anaerobic conditions. In effect, bokashi can be made with various combinations of resources, depending what is available regionally and financially. This makes bokashi far more affordable and accessible than commercial fertilizers.

Various studies have illustrated the benefits of using bokashi as a fertilizer to supply critical nutrients and promote plant growth (Álvarez-Solís et al. 2016; Aurora Gomez-Velasco et al. 2014; França et al. 2016; Lima et al. 2015; Peralta-Antonio et al. 2014). Because of bokashi's flexibility, each of the existing studies has made the fertilizer using different starting ingredients and slightly variable methodologies. While these studies have reported increased plant growth in response to bokashi application, few have compared the efficacy of bokashi made from different ingredients (Lima et al. 2015; Boechat, Santos, and Accioly 2013). Consequently, direct comparison of the types of bokashi, based on their starting ingredients, cannot be accurately made across studies due to an abundance of uncontrolled variables. Identifying how different ingredients influence bokashi quality could aid farmers in deciding which materials to use to optimize fertilization. To observe whether certain bokashi recipes are more beneficial to plant growth, I conducted a controlled study of the effects of different types of bokashi on crop growth.

1.2 Comparison of bokashi and compost

Bokashi is sometimes referred to as "bokashi *compost*." Both bokashi and traditional compost are nutrient amendments generated by the controlled breakdown of waste material, but the two products differ in their creation and composition. A comparative analysis of their effects on plant growth may elucidate whether the differences in the production of these organic amendments impacts their efficacy as soil amendments. In other words, this study aimed to compare the effects of compost and different types of bokashi on plant growth, while considering a developing mechanistic understanding of the utility of bokashi.

The process of making compost has been well documented in literature, while the relatively rapid transformation of bokashi has not been as extensively examined. Parallels in their transformations may exists, given that previous studies have shown that their effectiveness as soil amendments are comparable (Peralta-Antonio et al. 2014; Saldaña et al. 2014; Aurora Gomez-Velasco et al. 2014). During composting, temperature and moisture are manipulated to break down organic materials into a humus-like substance. Microorganisms utilize aerobic metabolism to transform things like manure, leaves, paper, and food waste into a product that resembles soil in consistency (Larney et al. 2006). Aeration is critical to composting to maintain aerobic conditions. Anaerobic microenvironments may arise, but it is estimated that these regions do not exceed 1% of total bacteria in compost (Ryckeboer et al. 2003). During composting, heat, carbon dioxide (CO_2), and water are released as byproducts of the aerobic breakdown of organic compounds.

While composting relies namely on aerobic processes, bokashi matures under partially anaerobic conditions to drive fermentation alongside both aerobic and anaerobic respiration. In the presence of plentiful oxygen, anaerobic processes are less frequent, as most facultative anaerobes preferentially select for the more energy efficient reduction of oxygen. In fact, various metabolic pathways may shut down in response to oxygen in the environment to promote full use of aerobic respiration (Wagner 2011; Giles et al. 2012; Berks et al. 1995; Hernandez and Rowe 1987).

Growing microbes need not just carbon as a source of energy, but also macronutrients including nitrogen, phosphorus, and potassium. Nitrogen is a critical component of the amino acids that make up proteins essential to all life. If N is deficient in a composting system, the decomposition process will slow down as microbial growth is stagnated. Unlike carbon, which is lost to the atmosphere as CO_2, N can be recycled within compost in forms that are not released to the atmosphere (Ryckeboer et al. 2003).

A major distinction between compost and bokashi is that compost requires extensive water management, during which piles must maintain moisture while still permitting oxygen flow required for aerobic processes (Larney et al. 2006). Decomposition processes occur mostly on liquid biofilms at the surfaces of organic particles. Biofilms are thin layers of microbes that stick to surfaces. If moisture is too low (below approximately 30%), microbial activity will slow down. On the other hand, if moisture is too high (above 65%), not only will bioavailable oxygen decrease, but nutrients may also leach from the system (Ryckeboer et al. 2003). In making bokashi, water is only added on the first day—when all ingredients are combined—to generate a moisture of approximately 60%, and then should not be added again. As microbial processes progress during maturation of both compost and bokashi, heat is released, and water will be produced by microbial respiration. Under covered bokashi piles, this moisture condenses, but in open compost systems, the water vapor is lost and moisture must be replenished.

1.2a Manure nutrients

Both bokashi and compost may be made from manure. Despite its status as agricultural waste, manure can serve as a virtually no- or low-cost, nutrient rich soil amendment. To reduce odors, fly-breeding, and the transferal of weed seeds and pathogens, manure is frequently composted before storage and application. Composting makes manure easier to handle, reduces waste volume, and generates a nutrient-rich fertilizer (Eghball et al. 2002). Manure is a source of inorganic nitrogen (N), in the forms nitrate (NO_3^-) and ammonium (NH_4^+), and upon application, organic N tends to mineralize. If the manure is first composted, the fraction of mineralized N will be low, as the most labile forms will quickly be converted under composting systems (Eghball et al. 2002). The advantage of this conversion during composting extends beyond producing a stable N pool upon amending soils, and also decreases the loss of N by runoff (Larney et al. 2006).

Early in the general composting process, most of the inorganic N is in the form of ammonium and very little appears as nitrate. As composting progresses, nitrification leads to the conversion of ammonium to nitrate, and thus the corresponding decrease in ammonium and increase in nitrate (Larney et al. 2006). In effect, the extent of maturity of composts can be measured by the ratio of NH_4^+ to NO_3^-, where the lower the ratio, the more stable the compost.

Most of the phosphorus contained in manure is inorganic and becomes immediately bioaccessible to plants following application. The fraction of available potassium (K) in manure is near unity, and substantial amounts of calcium (Ca) and magnesium (Mg) have also been reported (Eghball et al. 2002). N, P, K, Ca, and Mg are among the macronutrients, those required by plants in relatively large quantities (White and Brown 2010).

Disadvantages of composting manure include nutrient loss during the process, at approximately 20-40% total N and 46-62% total C losses (Eghball et al. 2002). In contrast, Larney et al. (2006) argues that total N concentrations remain constant when accounting for the reduced mass of matured composted. Much of the prowess of compost comes from its conversion of nutrients to stable forms, that can enrich soils with minimal runoff. In effect, much of the easily mineralizable N is converted to inorganic forms, while the remaining N remain in more stable forms. Although the NO_3^- and NH_4^+ are readily available to plants, upon application, NH_4^+ may be converted to ammonia (NH_3), and then lost to the atmosphere (Ryckeboer et al. 2003). This tendency for nitrogen to volatize as NH_3 decreases with increasing pH (Larney et al. 2006). Furthermore, composting manure results in the loss of C in the form of CO_2 (Eghball et al. 2002). P losses during composting are typically low and its concentration may even increase given the reduction in mass of composted material (Larney et al. 2006). Overall, remaining elemental nutrients in composted manure exist in a range of forms, and are overall less immediately bioaccessible to plants than those in unprocessed manure (Larney et al. 2006).

1.2b: Bokashi transformation and nutrients

As observed in *Chapter 2: An analysis of the chemical transformation of bokashi fertilizers*, the ammonium and phosphate levels of completed bokashi piles were high relative to those of soil. The nitrate levels, on the other hand, were very low. Consequently, ammonium to nitrate ratios in matured bokashi were very high, in contrast to the lower ratios of compost that reflect the stability of matured compost. While composting processes are characterized by the conversion of ammonium to nitrate and a decrease in N mineralization (Larney et al. 2006; Eghball et al. 2002), bokashi maturation appears to be driven by increases in N mineralization and the subsequent ammonium concentrations; meanwhile, nitrate levels remain relatively consistent. Both ammonium and nitrate—as well as soluble organic compounds—are plant-available forms of N.

Phosphate composition over bokashi maturation decreased over time and this may reflect the interconversion of phosphate from organic to occluded forms that ultimately create stable pools of phosphate in bokashi systems. P losses during composting are typically very low (Larney et al. 2006), but the forms in which the P are found in compost are not as known (Sinaj, Traore, and Frossard 2002). Estimates predict that most (40-77%) of the total P in compost is in slowly exchangeable or occluded inorganic P, while 2-16% of P in compost is in the form of readily exchangeable inorganic P (Frossard et al. 2002).

With regards to the different types of bokashi studied, the nutrient content of bokashi did not vary significantly when raw or smoked rice hulls were utilized, or when yeast or indigenous microorganisms (IMOs) were added to piles.

1.3 Experimental goals and hypothesis

Alone, an analysis of the nutrient content in bokashi extracts does not explain the efficacy of bokashi as a fertilizer. Indeed, the application of excessive nutrient concentration can be harmful to plant growth (Rivera 2001). In combination with nutrient levels identified in Chapter 2, understanding the potential benefits of bokashi as a soil-amendment requires measurements on the effects of bokashi treatments on crop growth. I sought to (1) quantify the effects of bokashi on plant growth and (2) examine whether different bokashi ingredients—charcoaled rice hulls versus raw rice hulls, yeast starter microbes versus IMO starter microbes—have an impact on plant productivity. To address these goals, I compared the growth responses of kale (*Brassica napus* subsp. *pabularia* - Red Russian) and cucumber (*Cucumis sativus* - Green Finger) in soils amended with bokashi versus those amended with compost or left unamended.

Given the high ammonium and phosphate content of bokashi, I hypothesized that plants grown in bokashi fertilizer will exhibit higher chlorophyll content than plants grown in compost or soil alone, because the strong correlation between chlorophyll levels and amounts of nitrogen in leaves has been well demonstrated (Clevers and Gitelson 2013; Baret, Houlès, and Guérif 2007; Vos and Bom 1993; Yoder and Pettigrew-Crosby 1995). I also hypothesized that plant productivity—as measured by plant height, length of longest leaf, and dry biomass—in bokashi-amended soils will be comparable to that of those grown in compost amended soil. Moreover, given the lack of variability in nutrient levels across the different types of bokashi, I hypothesize that there will be little variation in the effects of the different types of bokashi on plant growth.

2. Methods

2.1 Experimental set-up

To analyze the efficacy of bokashi as a fertilizer for crops, I conducted a greenhouse experiment in which kale (*Brassica napus* subsp. *pabularia* - Red Russian) and cucumber (*Cucumis sativus* - Green Finger) seedlings were treated with various types of bokashi, compost, or the control condition of soil alone. I chose to study kale and cucumber to represent plants of which people consume the leaves and fruit, respectively. The bokashi treatments applied were the bokashi with raw rice hulls (BR), charred rice hulls (BC), yeast starter cultures (BY), and indigenous microorganism (IMO) starter cultures (BI), made as described in Chapter 2. The compost was Miracle Grow's "Nature's Care: Really Good Compost," made from some combination of forest, manure, mushroom, and food waste compost. The base soil was "Coast of Maine Topsoil" made from loam, compost, and peat (the same soil that was used to make the different types of bokashi).

I started kale and cucumber seedlings in plug trays with seed starting mix. Approximately 3-weeks after germination, I transplanted cucumber and kale seedlings into 400-mL and 500-mL pots, respectively, with one seedling per pot. Each pot contained one handful of either bokashi, compost, or soil, between two handfuls of soil. In effect, I created a soil-barrier between the amendments and the seedlings to avoid nutrient burn (the overloading of plants with nutrients) (Rivera 2001). I measured in handfuls to replicate how farmers and gardeners would apply fertilizer or compost. A handful amounted to approximately 60 grams for dry bokashi, 80 grams for moist compost, and 140 grams for moist soil. I transplanted 1 seedling per pot, with each of the following 6 treatments replicated 8 times per species: BC, BR, BY, BI, compost, and soil. In total, there were 96 pots: 2 species × 6 treatments × 8 replicates. Within the 8 replicate pots, each of the 4 original bokashi replicate piles were represented twice. Plants were watered as needed and received ambient light.

Moreover, I measured the phosphate, nitrate, and ammonium levels of the compost using the Astoria-Pacific Discrete Analyzer (Astoria-Pacific, Inc., Clackamas, OR, USA), and compared the values to those for the different types of bokashi and soil, as described in Chapter 2. Finally, it should be noted that both the bokashi piles made with raw rice hulls (BR) and those made with the yeast starter microbe (BY) are identical in recipe, but were made at different time points with manure sourced at a different time.

2.2 Data collection and statistics

I measured plant height, longest leaf length, and the number of leaves for each plant approximately once a week for three weeks. Plant heights were measured as the extension of the stem from soil-level to the base of the highest leaf. Leaf lengths were measured from the leaf base to the apex, and the lengths of only the longest leaves were recorded. Only true leaves—and not cotyledons (the first leaves to appear from germinating seeds)—were counted. "Week 1" measurements were taken a few days following transplantation, to represent a baseline value for plant growth, given the variation in seedling dimensions.

Shortly before harvesting full plants (3 weeks after seeds were sown), I measured the chlorophyll levels of two leaves per plant using the Opti-Sciences CCM-300 Chlorophyll Content Meter (values are expressed as mg m^{-2}, using a generic calibration). I chose to examine the newest, fully matured leaves. I harvested and dried aboveground tissue in a 65˚C oven for approximately 2 days before measuring them for dry biomass, using an analytical balance.

Analysis of Variance (ANOVA) and were used to examine variation in responses variables based on soil amendment types—bokashi treatment type, compost, and soil—and their interaction. The Tukey Test was used to look for pair-wise differences among amendment types. Analyses were performed using R (R Core Team 2014), with the 'ez' package (Lawrence 2016) used for repeated measures ANOVA.

3. Results

3.1 Amendment effects on plant growth

Plant heights, longest leaf lengths, dry biomass, and chlorophyll levels—for both kale and cucumber plants—were significantly higher for plants treated with bokashi fertilizers than for those receiving compost or soil alone.

The average heights for both kale and cucumber plants treated with bokashi—following week 1—were higher than those of compost-treated soils and the control (Figure 1). For kale, plants in compost-treated soils exhibited slightly taller heights (Figure 1a), whereas the heights of cucumber plants in compost-treated and untreated soils were nearly identical (Figure 1b). A closer examination of plant heights for kale and cucumber grown in different types of bokashi demonstrates that the tallest plants were grown in soils treated with BC (Figure 2). The interactions between treatment and time were significant for both crop species.

Similarly, longest leaf lengths were significantly longer for both kale and cucumber plants treated with bokashi (Figure 3), and for kale plants—to a much greater extent than cucumber plants—the leaf lengths were especially longer for kale plants treated with BC (Figure 4). The lengths of leaves on plants treated with compost or grown in soil alone were nearly identical. The interactions between treatment and time were significant for both crop species.

Leaf counts were relatively similar across treatments. For kale plants, most plants began with 2-3 leaves, then grew to 3-6 leaves, and then to 3-7 leaves, with plants grown in bokashi exhibiting the highest leaf counts. For cucumber plants, most plants began with 1-2 leaves, then grew to 2-4 leaves, and then 3-5 leaves, with plants grown in bokashi exhibiting the highest leaf counts.

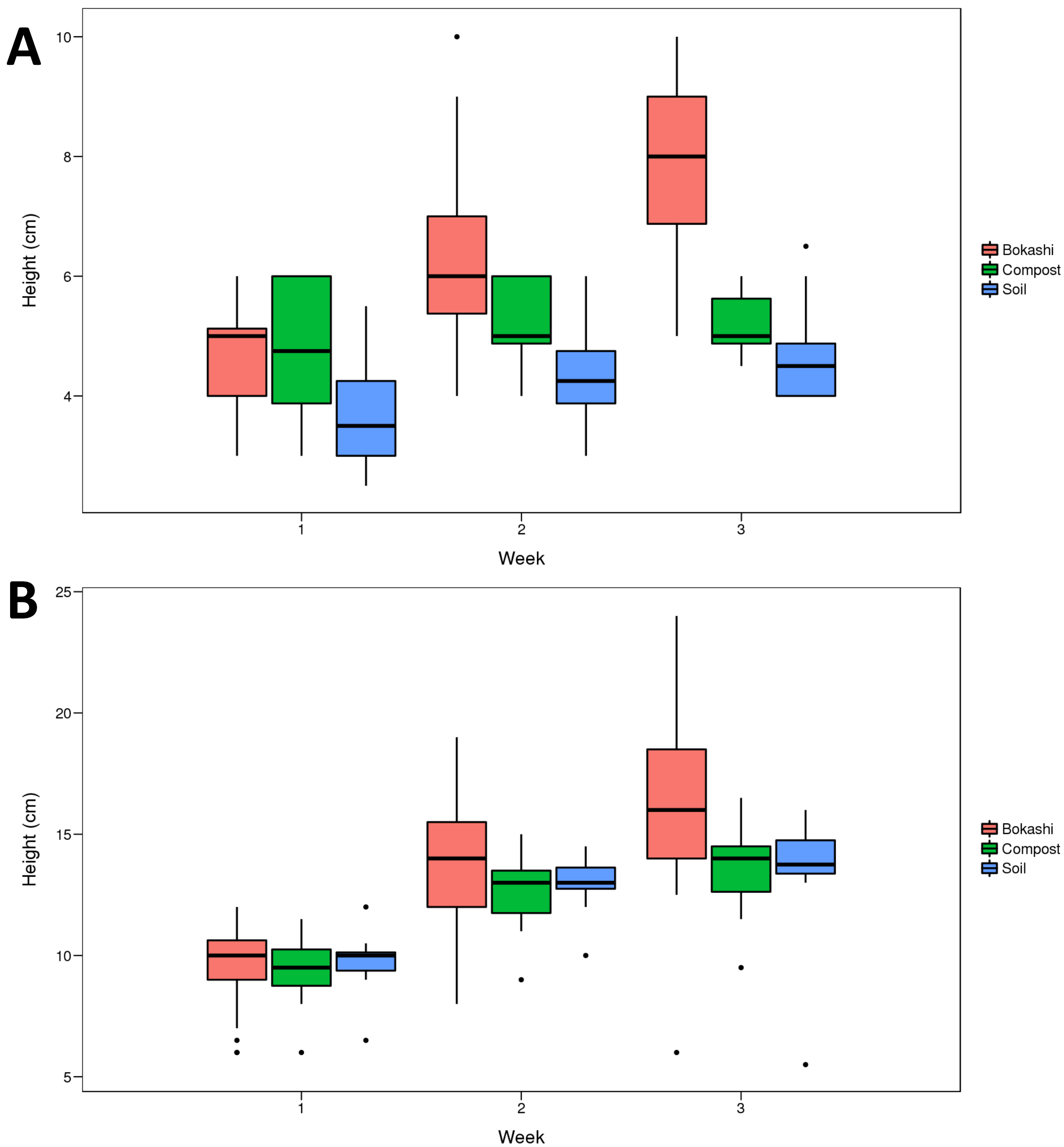

Figure 1: Mean plant heights following treatment with bokashi or compost. The heights of (a) kale and (b) cucumber plants were measured over three weeks following transplantation in soils amended with various types of bokashi, or compost, or soil alone.

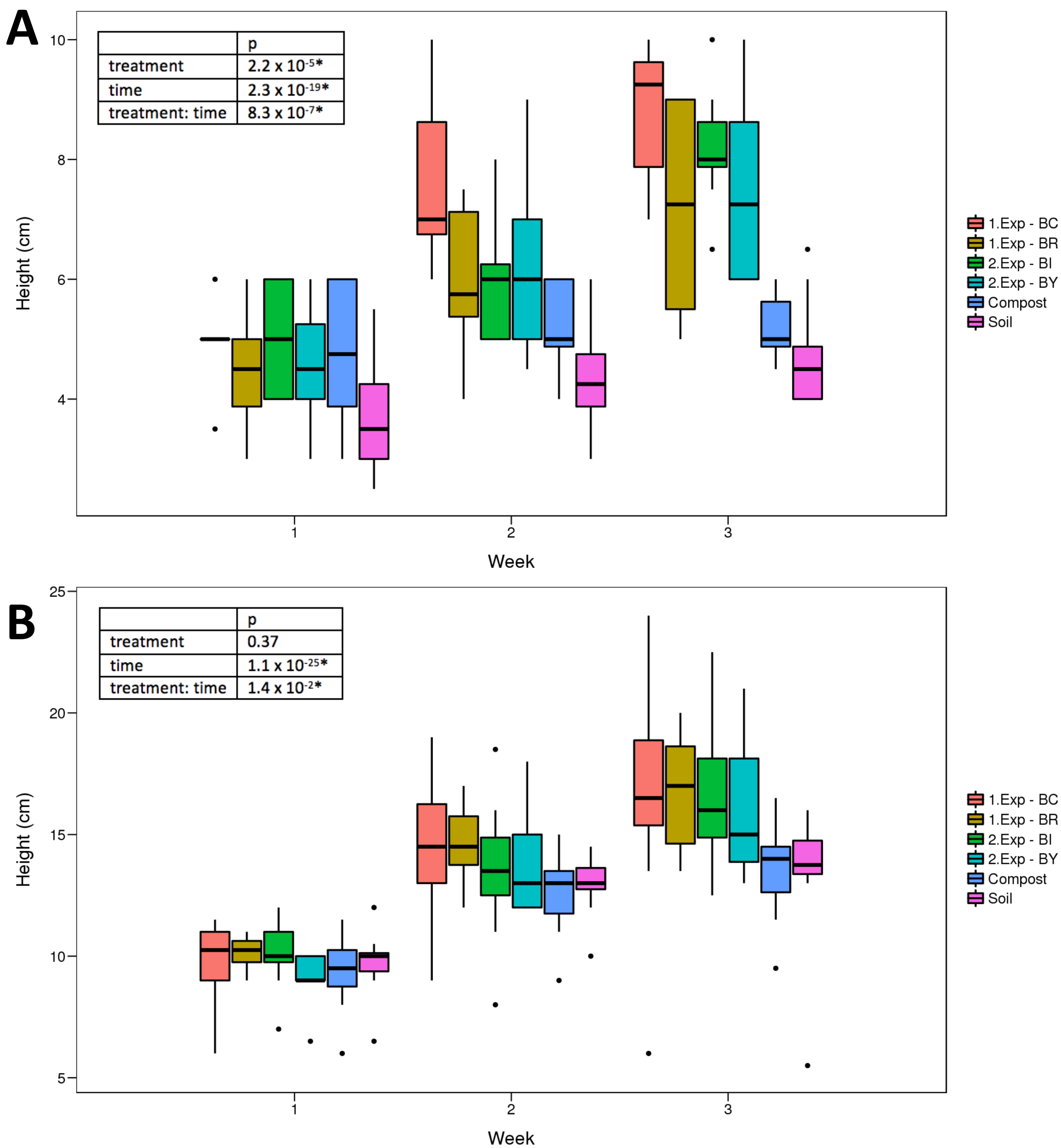

Figure 2: Plant heights following treatment with various types of bokashi or compost. The heights of (a) kale and (b) cucumber plants were measured over three weeks following transplantation in soils amended with various types of bokashi (BC and BR, made in experiment 1; BI and BY, made in experiment 2), or compost, or soil alone.

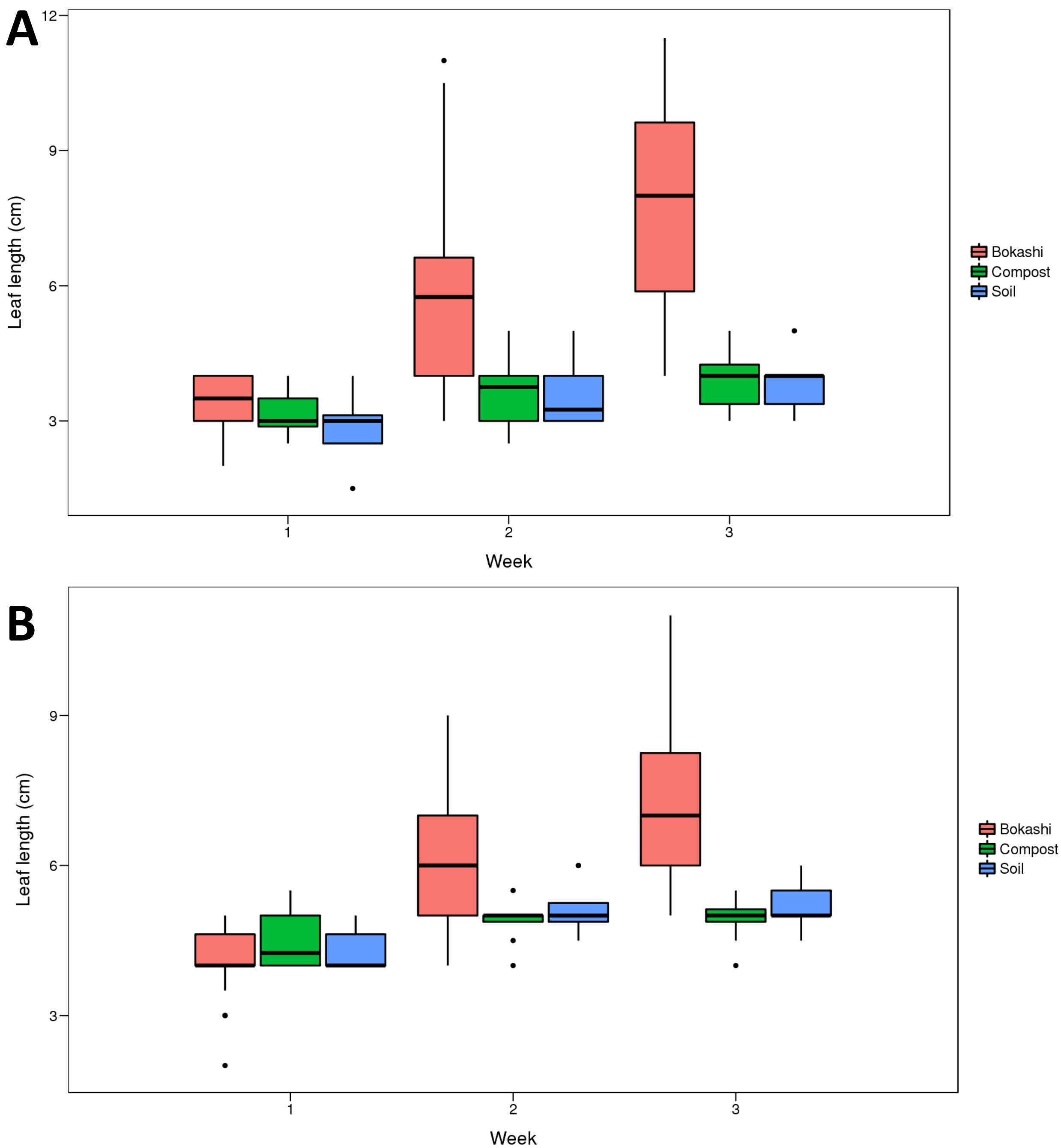

Figure 3: Mean longest leaf lengths following treatment with bokashi or compost. The of the longest leaves of (a) kale and (b) cucumber plants were measured over three weeks following transplantation in soils amended with various types of bokashi, or compost, or soil alone.

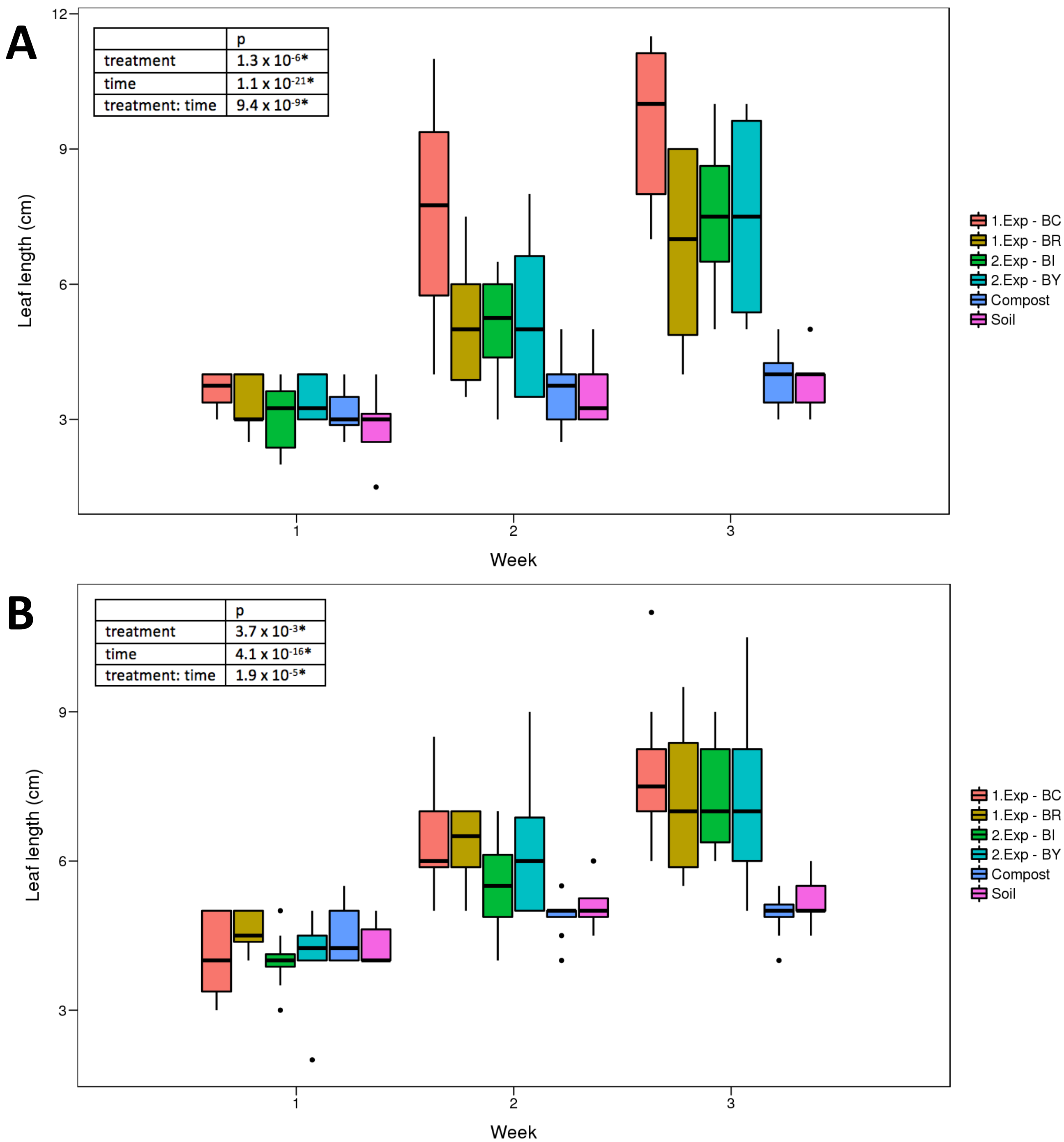

Figure 4: Plant longest leaf lengths following treatment with various types of bokashi or compost. The heights of (a) kale and (b) cucumber plants were measured over three weeks following transplantation in soils amended with various types of bokashi (BC and BR, made in experiment 1; BI and BY, made in experiment 2), or compost, or soil alone.

Dry biomass values for both kale and cucumber plants were significantly higher for plants grown in bokashi-amended soils than for those grown in compost-amended or untreated soils (Figure 5). As is consistent with plant height and longest leaf lengths, plants treated with BC exhibit the greatest dry biomass, for both kale and cucumber plants. Biomasses under the all treatments were statistically distinct, but biomasses of plants grown in bokashi were not significantly different from each other. The biomass of crops under the compost and control treatments were not significantly different (Appendix B).

Chlorophyll levels for leaves on kale and cucumber plants treated with bokashi were significantly higher than those of plants treated with compost or untreated (Figure 6). For kale plants, charcoal-bokashi treated plants exhibited the highest chlorophyll levels (Figure 6a). Chlorophyll levels under the all treatments were statistically distinct, but chlorophyll of plants grown in bokashi were not significantly different from each other. The chlorophyll concentrations of crops under the compost and control treatments were not significantly different. Figure 7 presents a visual comparison of kale and cucumber plants grown in charcoal-bokashi, compost, and the control condition.

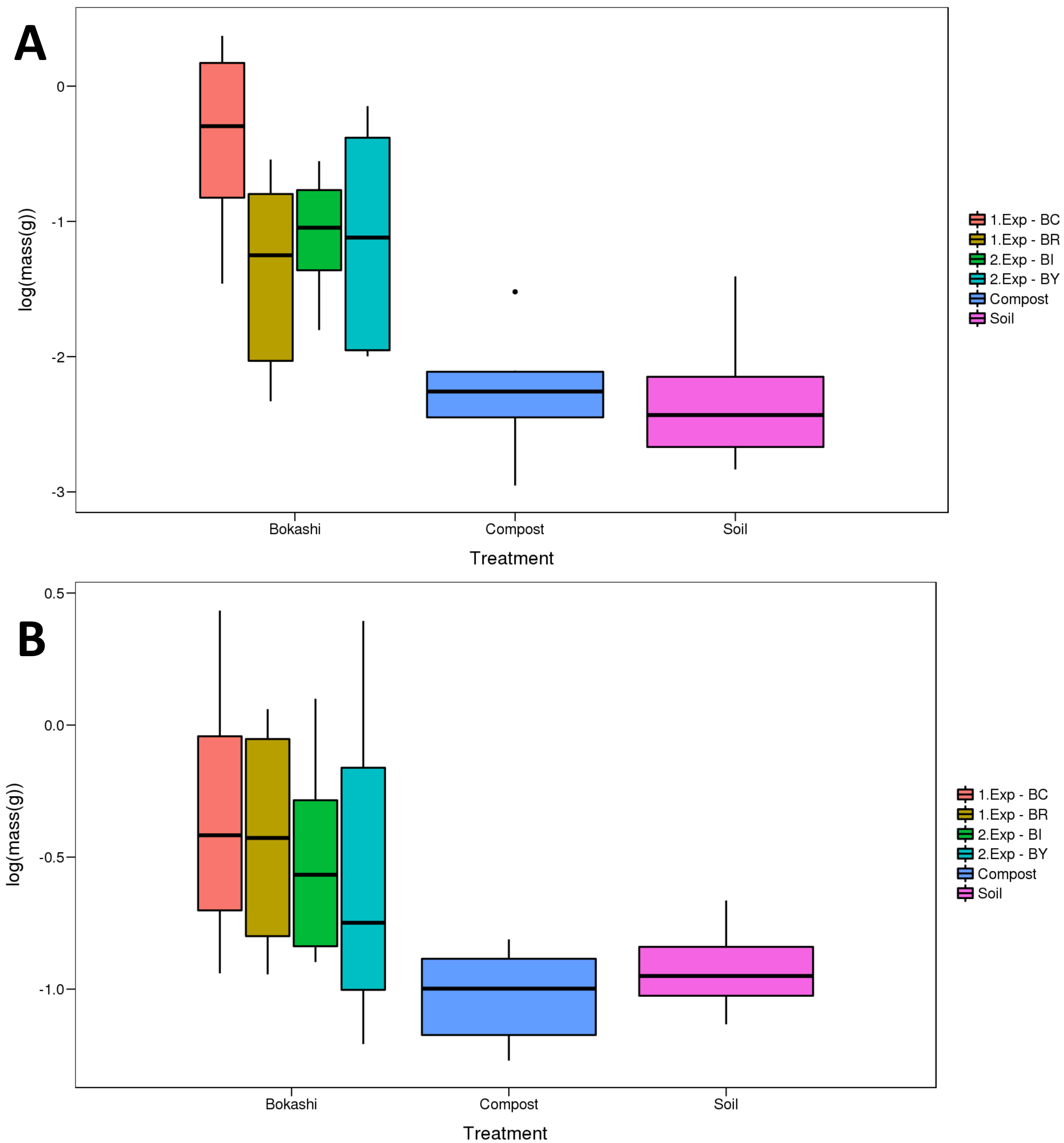

Figure 5: Plant dry biomass following treatment with various types of bokashi or compost. The dried biomass of (a) kale and (b) cucumber plants were measured after three weeks of growth in soils amended with various types of bokashi (BC and BR, made in experiment 1; BI and BY, made in experiment 2), or compost, or soil alone. Data is $\log_{10}$-transformed.

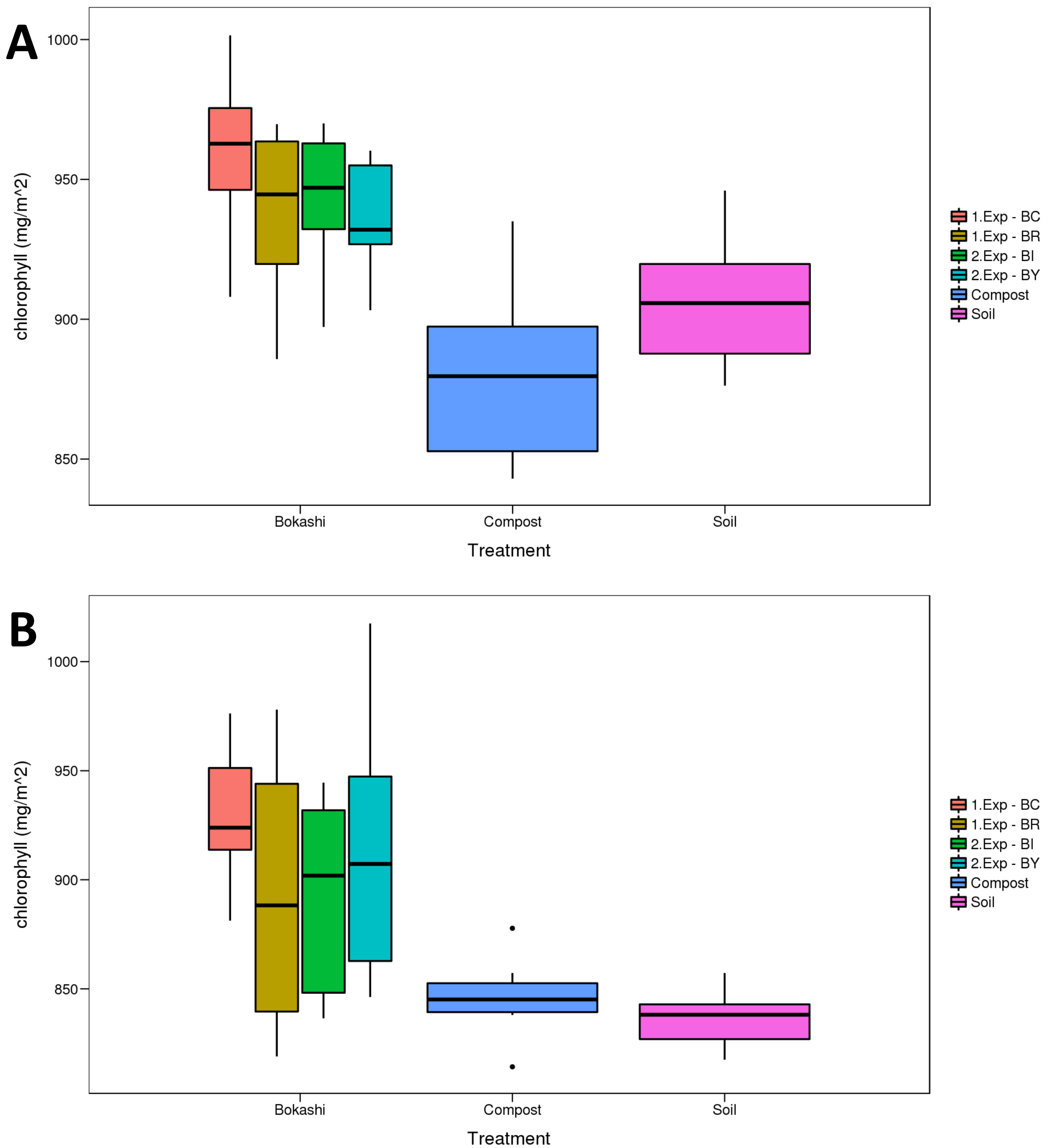

Figure 6: Figure 6: Leaf chlorophyll levels during treatment with various types of bokashi or compost. The chlorophyll concentrations of (a) kale and (b) cucumber leaves were measured after three weeks of crop growth in soils amended with various types of bokashi (BC and BR, made in experiment 1; BI and BY, made in experiment 2), or compost, or soil alone.

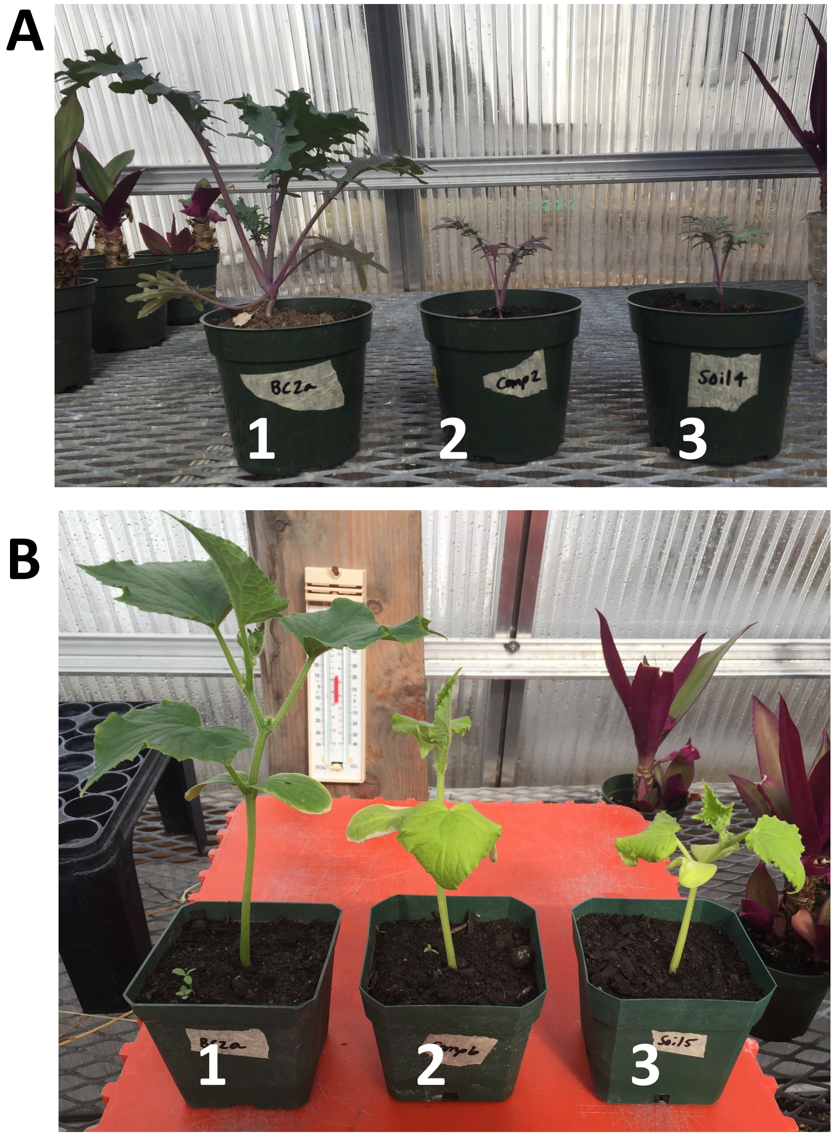

Figure 7: Visual comparison of plant growth across treatments. (a) Kale and (b) cucumber plants following three weeks of growth in soils amended with (1) bokashi-charcoal, (2) compost, (3) no amendment.

3.2 Interactions between plant growth and bokashi nutrient composition

The results of the nutrient composition study described in Chapter 2 in relation to the growth of the crops in the present study illustrate significant trends in bokashi nutrient composition and plant growth.

There is a significant positive correlation between chlorophyll concentrations of crop leaves and dry plant biomass for both cucumber and kale plants grown in bokashi-treated soils (Figure 8). The correlation between the ammonium concentrations of fully matured bokashi and the chlorophyll levels of cucumber leaves is also positive; this correlation is significant for cucumber plants but not for kale plants, although the relationship tends toward positivity (Figure 9). Conversely, the positive correlation between bokashi ammonium concentrations and biomass was significant for kale plants but not cucumber plants, although the relationship also tended toward positivity (Figure 10). Although not statistically significant, there appeared to be a negative correlation between final phosphate concentrations of bokashi and crop biomass (Figure 11).

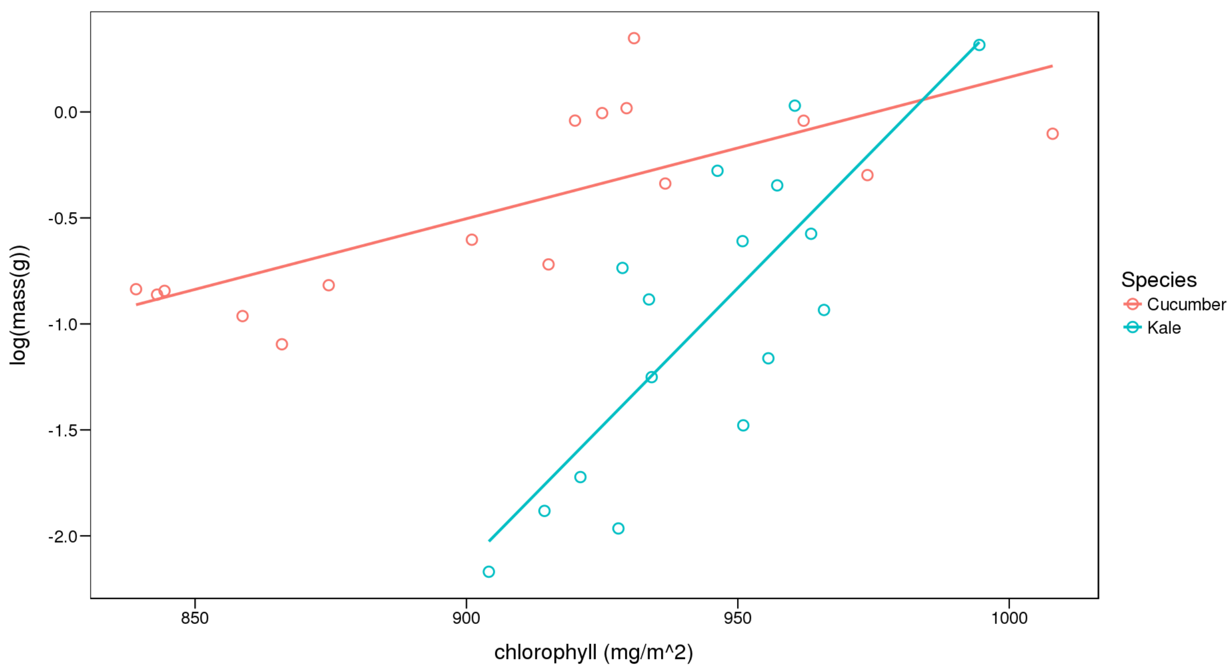

Figure 8: Positive correlation between chlorophyll concentrations and plant biomass. Mean chlorophyll concentrations of leaves of cucumber and kale plants and dry biomass measured 3 weeks after growing in soils amended with different types of bokashi are plotted. Lines represent linear regressions. Biomass values are $\log_{10}$ transformed.

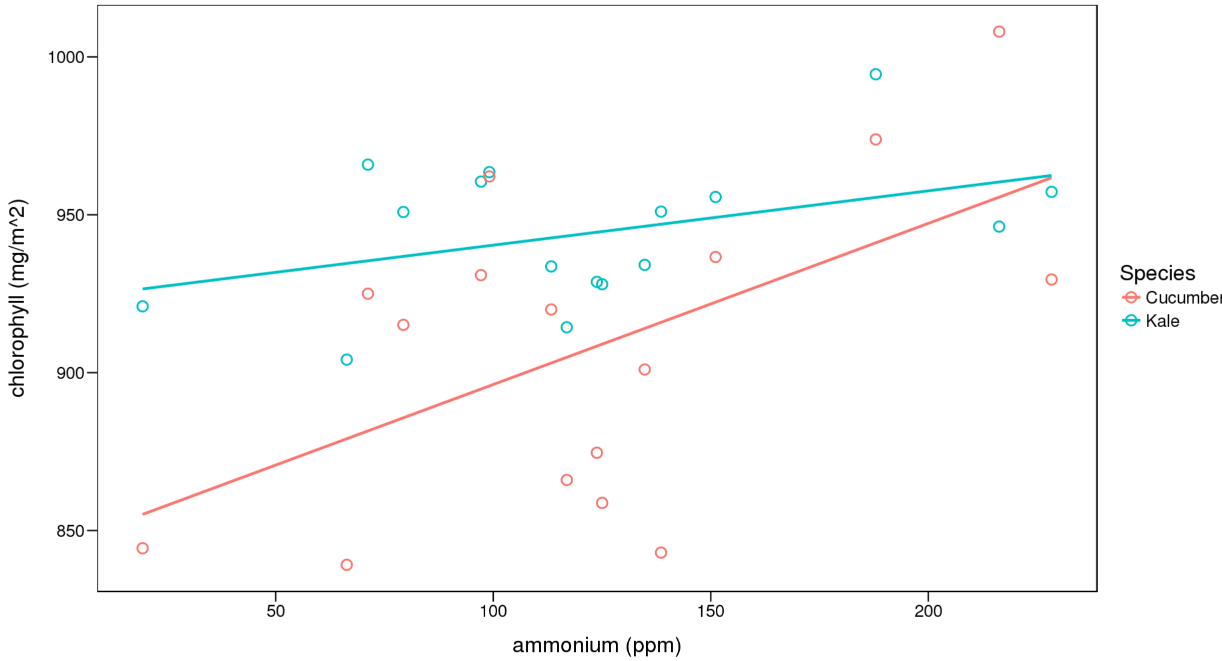

Figure 9: Positive correlation between ammonium concentrations of bokashi and chlorophyll concentrations in cucumber and kale leaves. Mean final ammonium concentrations of all bokashi types (by replicate) and chlorophyll concentrations of leaves of cucumber and kale plants 3 weeks after growing in soils amended with different types of bokashi are plotted. Lines represent linear regressions.

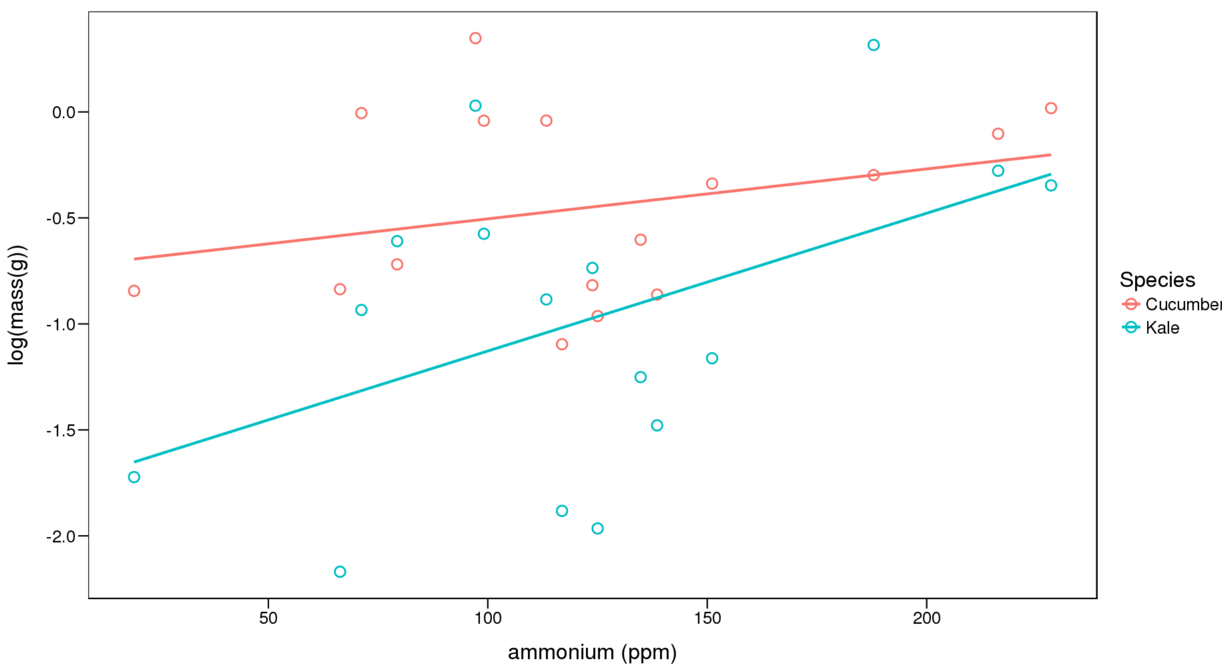

Figure 10: Positive correlation between ammonium concentrations and plant biomass. Mean final ammonium concentrations of all bokashi types (by replicate) and chlorophyll concentrations and dry biomass collected 3 weeks after growing in soils amended with different types of bokashi are plotted. Lines represent linear regressions. Biomass values are $\log_{10}$ transformed.

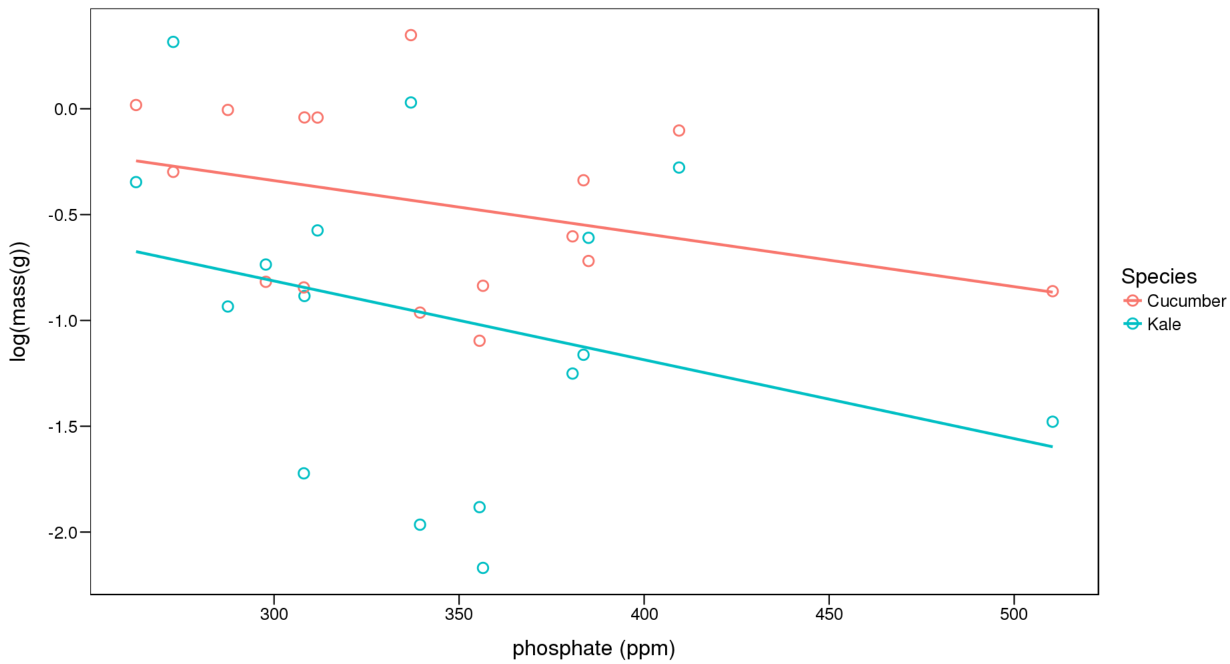

Figure 11: Negative correlation between phosphate concentrations and plant biomass. Mean final phosphate concentrations of all bokashi types (by replicate) and chlorophyll concentrations and dry biomass collected 3 weeks after growing in soils amended with different types of bokashi are plotted. Lines represent linear regressions. Biomass values are $\log_{10}$ transformed.

4. Discussion

4.1 Analysis of increased crop growth in bokashi-amended soils

As hypothesized, plants treated with bokashi exhibited heightened growth and chlorophyll levels relative to unamended soils. Moreover, bokashi outperformed compost in increasing plant growth, with respect to height, length of longest leaf, chlorophyll concentration, and dry biomass. These results were consistent with a number of studies illustrating—upon the application of bokashi—improved plant height, number of leaves, and/or biomass of tomatoes (França et al. 2016), peppers and onions (Álvarez-Solís et al. 2016), coffee (Aurora Gomez-Velasco et al. 2014), *Pinus pseudostrobus* trees (Jaramillo-López, Ramírez, and Pérez-Salicrup 2015), and mango cultivars (Peralta-Antonio et al. 2014).

There were minimal differences in the extent to which different types of bokashi increased plant growth, with BC consistently exhibiting the greatest increases in growth relative to control and compost conditions, but not significantly different than other bokashi types tested. The compost did not significantly improve plant growth over soil alone, and often exhibited nearly identical growth patterns as soil alone. Compost may be produced by a variety of inputs and variable processes that may influence amendment efficacy, thus, the compost utilized in this study is not intended to be representative of compost performance as an amendment. Rather, the scope of this study illustrates the advantages of using bokashi as an amendment to enhance crop growth.

Early biomass, height, and leaf area have been identified as predictors of subsequent crop growth and competitive fitness against weeds, a prevalent burden in organic agricultural systems (Acciaresi, O. Chidichimo, and Sarandón 2001). Moreover, dry biomass and leaf area have been directly correlated with leaf photosynthetic rates, and leaf chlorophyll concentrations are important indicators of crop N (Zhao et al. 2005). In effect, the increases in cucumber and kale biomass, leaf length, height, and chlorophyll concentrations upon soil amendment with bokashi, in just the first three weeks of growth may be reflective of increased crop productivity.

4.2 Developing a mechanistic understanding of bokashi fertilization

Combining a nutrient analysis of bokashi and the effects of the amendment on plant growth shed insight into the mechanistic links between bokashi composition and efficacy. There was a positive correlation between ammonium concentrations in mature bokashi piles and chlorophyll concentrations of leaves of plants treated with bokashi. This suggests that increased ammonium composition of bokashi fertilizers may increase plant chlorophyll, the pigment critical for absorbing the energy required for photosynthesis, which, in turn, would increase plant growth. The positive correlation between leaf chlorophyll concentrations and plant biomass further supports this pathway for enhanced plant growth by bokashi bioavailable N contribution.

The negative correlation between bokashi phosphate concentrations and crop biomass was not significant. Phosphorus is a critical plant macronutrient, and while phosphate concentrations appeared to decrease over bokashi maturation, the results of this study suggest that such a decrease in phosphate concentrations would not negatively impact plant growth. Furthermore, declines in phosphate concentrations do not capture the interconversion of phosphate between organic and occluded reservoirs as chemical reactions and microbial processes cycle nutrients.

5. Conclusion

Bokashi is an effective soil amendment, as its contribution of the macronutrient N has been linked to increased chlorophyll concentrations and subsequent increases in plant biomass for both kale and cucumber plants. Different types of bokashi—regardless of the form of organic material or added microbes—contribute similar amounts of nutrients and, in effect, improve plant growth to the same degree. Thus, the advantageous of bokashi are not only in its nutrient composition and efficacy, but also the flexibility of materials that may be combined to create the productive fertilizer.

Conclusions: An agroecological approach

1. Bokashi: recommendations for field application

Fostering sustainable food systems requires the development of agroecologically sound natural resource management approaches to ensure that food is accessible to the hungry, agricultural systems support farmer livelihoods, and farming practices are environmentally sustainable (Méndez, Bacon, and Cohen 2013). For such agroecological approaches to succeed, they must increase farm productivity, prove environmentally and economically sustainable, and be adaptable to various climates and contexts (Altieri 2002). Bokashi fits within the framework of a sustainable agroecological technique as it improves crop growth, diverts waste streams, and can be made with various ingredients without compromising fertilizer efficacy.

From start to finish, bokashi takes approximately two weeks to mature and requires shelter from precipitation and a management process that involves covering and aerating piles daily. After maturation, bokashi contains a wealth of nutrients, including an abundance of mineralized nitrogen in the bioavailable form of ammonium, which reduces common nutrient limitations in agricultural systems. Nitrogen mineralization during bokashi maturation presents a pathway for breaking down manure and other organic material into a supply of nitrogen that increases chlorophyll concentrations in plant tissue and, in effect, increases crop biomass and agricultural yield.

Moreover, the controlled study of different types of bokashi—those with charcoal versus raw rice hulls, or yeast versus IMO starter microbes—illustrates little variation in nutrient composition or fertilizer efficacy of bokashi. In effect, farmers may save money by not purchasing baker's yeast and instead harvest IMOs from local soils; and save time and resources by using raw rice hulls, rather than charcoal hulls. In summary, the flexibility of bokashi renders it adaptable to various climates and contexts, in which farmers can make bokashi based on accessible ingredients, without compromising the qualities of the amendment.

2. Bridging knowledge systems

I have assessed analytically the properties of bokashi in an attempt to overcome the challenge of high uncertainty in our understanding of complex ecosystems as a barrier to combining scientific theory and native knowledge (Vandermeer and Perfecto 2013). I sought to pull discussions of sustainable agroecological techniques based on traditional knowledge into Western science and agricultural systems in the USA. However, I have found two major challenges to these frameworks:

First, even after examining the biochemical transformations of bokashi, the complex interactions of biological and chemical processes underlying the conversion of waste material into a productive fertilizer are largely unknown. Rather, among the most meaningful interpretations of this study is that the controlled-decomposition process produces an effective fertilizer regardless of variable starting ingredients—an observation that was clearly made and shared when bokashi was first developed. This characteristic of bokashi is one of its greatest advantages and perhaps among the main reasons it has been adapted from East Asia to Latin America, granting a traditional technique great breadth.

Second, to delve deeply into the mechanisms of a traditional agroecological technique, like bokashi, without maintaining the bidirectional exchange of knowledge risks contributing to epistemic injustice, the exclusion of those originally sourcing the knowledge from participating in its dissemination. This injustice maintains the divide between sources of knowledge and hinders the potential for synergistic collaboration (Fortmann 2008; Fricker 2009).

To avoid these negative consequences, agroecological research must be done by directly collaborative interdependent science, one in which conventional scientists—those with formal education and training—and civil scientists—those who develop knowledge in various, non-formalized methods—partner as equals in developing more complete knowledge systems (Fortmann 2008; Méndez, Bacon, and Cohen 2013). In this framework, I characterize the progress I have made in this study as a contribution to an early stage of this approach. Operating by institutionalized research, I cannot fully bridge gaps in knowledge systems, but can contribute to identifying and approaching them. Overcoming the challenges of making bokashi on a suburban college campus in the winter lead me to creatively imagine novel ways in which bokashi processing could be adapted to different contexts. Additionally, the conclusions of this study reaffirming the flexibility of bokashi have expanded the reaches of this traditional agroecological technique, further scaling out the application of the technique. Moreover, with the massive amounts of agricultural waste, especially manure, produced in the USA, bokashi presents a rapid, low-cost—although somewhat high labor—outlet for diverting waste streams into a productive fertilizer.

The next stage of this interdependent approach involves not solely cycling these findings in academic settings and publications, but partnering directly with local farmers to further adapt and study agroecological techniques like bokashi, without erasing their origins and value as traditional agroecological techniques. This agroecological approach could bridge gaps in knowledge systems by broadening deeply explored, but highly locally-relevant, field knowledge, and substantially increasing food production by implementing techniques that already exist and may be adapting (IAASTD 2009; De Schutter 2010; Altieri, Funes-Monzote, and Petersen 2012). Fostering and sustaining this bidirectional exchange of information will be challenging, but no amount of scientific study will promote food sovereignty without equitable and sustained partnerships with the people most immediately involved in cultivating food systems.

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Appendix

Appendix A: Microbial analysis

A microbial analysis was designed to gain insight into the microbial communities driving the biochemical transformation of bokashi from starting materials into a mature product. Metagenomic profiling of bokashi communities are in the process of sequencing.

To prepare for metagenomic profiling, frozen samples were thawed before DNA extraction and isolated using the Qiagen DNeasy PowerSoil kit, per the manufacturer's instructions. After extraction, DNA concentrations were measured using the Thermo Scientific, Inc. NanoDrop. Extracts were stored at -80˚C prior to sending to Integrated Microbiome Resource (IMR) at the Centre for Comparative Genomics and Evolutionary Bioinformatics (CGEB) at Dalhouise University for metagenomics profiling using amplicon targets 16S to identify bacterial and archaeal sequences and amplicon fungi-specific ITS.

Aside from metagenomic data, I observed various indicators of microbial growth on maturing bokashi piles, including white filamentous fungi, blue-green growth, tan string-like microbes, and yellow growth (Figure B1).

Figure B1: Observed microbial growth on bokashi piles: (A) filamentous, white fungi, (B) and (C) blue-green growth, (D) tan string-like microbes, (E) bright yellow growth.

Appendix B: Statistics

Expanded results of ANOVA and Tukey Tests
* p <0.05

Ammonium

	DFn/DFd	p
Treatment	3/12	2.410437e-01
Time	4/48	0.0003082705*
Treatment: Time	12/48	0.0058090091*

```
         diff        lwr       upr     p adj
I-C   6.89197  -81.74175  95.52569 0.9954265
R-C -60.80722 -149.44094  27.82650 0.2284344
Y-C  46.82129  -41.81243 135.45500 0.4308312
R-I -67.69919 -156.33291  20.93453 0.1605249
Y-I  39.92932  -48.70440 128.56303 0.5583600
Y-R 107.62850   18.99479 196.26222 0.0164623
```

Nitrate

	DFn/DFd	p
Treatment	3/12	0.41876928
Time	4/48	0.72093226
Treatment: Time	12/48	0.06014575

```
          diff       lwr       upr     p adj
I-C -1.0964286 -3.148845 0.9559882 0.4216676
R-C -1.4435714 -3.495988 0.6088454 0.2115889
Y-C -1.6071429 -3.659560 0.4452740 0.1466524
R-I -0.3471429 -2.399560 1.7052740 0.9570139
Y-I -0.5107143 -2.563131 1.5417025 0.8796235
Y-R -0.1635714 -2.215988 1.8888454 0.9950819
```

Phosphate

	DFn/DFd	P
Treatment	3/12	1.209837e-01
Time	4/48	4.898740e-11*
Treatment: Time	12/48	1.662693e-02*

```
         diff       lwr       upr     p adj
I-C  31.15127 -105.0870 167.38954 0.9031294
R-C -27.90816 -164.1464 108.33011 0.9275243
Y-C  -2.06816 -138.3064 134.17011 0.9999651
R-I -59.05943 -195.2977  77.17884 0.5875880
Y-I -33.21943 -169.4577 103.01884 0.8856813
Y-R  25.84000 -110.3983 162.07826 0.9411155
```

pH

	DFn/DFd	p
Treatment	3/12	0.3829336226
Time	4/48	0.001547451*
Treatment: Time	12/48	0.100466974

```
diff        lwr       upr      p adj
I-C -0.2250 -0.6391081 0.1891081 0.4078398
R-C -0.0375 -0.4516081 0.3766081 0.9928394
Y-C  0.0350 -0.3791081 0.4491081 0.9941530
R-I  0.1875 -0.2266081 0.6016081 0.5544578
Y-I  0.2600 -0.1541081 0.6741081 0.2928613
Y-R  0.0725 -0.3416081 0.4866081 0.9527100
```

Kale heights

	DFn/DFd	p
Treatment	5/42	2.154628e-05*
Time	2/84	2.303454e-19*
Treatment: Time	10/84	8.334310e-07*

Cucumber heights

	DFn/DFd	p
Treatment	5/42	3.698871e-01
Time	2/84	1.136044e-25*
Treatment: Time	10/84	1.365986e-02*

Kale longest leaf length

	DFn/DFd	P
Treatment	5/42	1.340991e-06*
Time	2/84	1.099961e-21*
Treatment: Time	10/84	9.375924e-09*

Cucumber longest leaf length

	DFn/DFd	P
Treatment	5/42	3.715920e-03*
Time	2/84	4.106837e-16*
Treatment: Time	10/84	1.948755e-05*

Kale biomass

p = 3.54e-07 ***

```
                   diff        lwr         upr     p adj
BI-BC        -0.726374451 -1.6484278  0.19567893 0.1969464
BR-BC        -0.975626091 -1.8976795 -0.05357271 0.0325835*
BY-BC        -0.735882950 -1.6579363  0.18617043 0.1857367
Comp-BC      -1.885981729 -2.8080351 -0.96392834 0.0000040*
```

	diff	lwr	upr	p adj
Control-BC	-1.951959308	-2.8740127	-1.02990592	0.0000020*
BR-BI	-0.249251640	-1.1713050	0.67280174	0.9646603
BY-BI	-0.009508499	-0.9315619	0.91254489	1.0000000
Comp-BI	-1.159607278	-2.0816607	-0.23755389	0.0065560*
Control-BI	-1.225584858	-2.1476382	-0.30353147	0.0035409*
BY-BR	0.239743141	-0.6823102	1.16179653	0.9700696
Comp-BR	-0.910355638	-1.8324090	0.01169775	0.0547524*
Control-BR	-0.976333217	-1.8983866	-0.05427983	0.0323957*
Comp-BY	-1.150098779	-2.0721522	-0.22804539	0.0071534*
Control-BY	-1.216076359	-2.1381297	-0.29402297	0.0038740*
Control-Comp	-0.065977579	-0.9880310	0.85607581	0.9999340

Cucumber biomass

p = 0.00618 **

	diff	lwr	upr	p adj
BI-BC	-0.18447203	-0.7811928	0.412248700	0.9383855
BR-BC	-0.10127722	-0.6979980	0.495443507	0.9956445
BY-BC	-0.23509820	-0.8318189	0.361622525	0.8455602
Comp-BC	-0.69223364	-1.2889544	-0.095512916	0.0146989*
Control-BC	-0.58223389	-1.1789546	0.014486842	0.0594353
BR-BI	0.08319481	-0.5135259	0.679915536	0.9982861
BY-BI	-0.05062617	-0.6473469	0.546094554	0.9998471
Comp-BI	-0.50776162	-1.1044823	0.088959113	0.1356844
Control-BI	-0.39776186	-0.9944826	0.198958871	0.3650614
BY-BR	-0.13382098	-0.7305417	0.462899747	0.9843573
Comp-BR	-0.59095642	-1.1876772	0.005764306	0.0535840*
Control-BR	-0.48095666	-1.0776774	0.115764064	0.1774667
Comp-BY	-0.45713544	-1.0538562	0.139585288	0.2221287
Control-BY	-0.34713568	-0.9438564	0.249585046	0.5162792
Control-Comp	0.10999976	-0.4867210	0.706720487	0.9935975

Kale chlorophyll

p = 7.07e-06 ***

	diff	lwr	upr	p adj
BI-BC	-17.75000	-58.75397	23.253972	0.7875030
BR-BC	-21.37500	-62.37897	19.628972	0.6309065
BY-BC	-23.53125	-64.53522	17.472722	0.5311015
Comp-BC	-80.50000	-121.50397	-39.496028	0.0000090*
Control-BC	-54.12500	-95.12897	-13.121028	0.0038366*
BR-BI	-3.62500	-44.62897	37.378972	0.9998128
BY-BI	-5.78125	-46.78522	35.222722	0.9981916
Comp-BI	-62.75000	-103.75397	-21.746028	0.0005759*
Control-BI	-36.37500	-77.37897	4.628972	0.1080436
BY-BR	-2.15625	-43.16022	38.847722	0.9999857
Comp-BR	-59.12500	-100.12897	-18.121028	0.0012967*
Control-BR	-32.75000	-73.75397	8.253972	0.1850871
Comp-BY	-56.96875	-97.97272	-15.964778	0.0020815*
Control-BY	-30.59375	-71.59772	10.410222	0.2472225
Control-Comp	26.37500	-14.62897	67.378972	0.4045667

Cucumber chlorophyll
p = 0.000313 ***

	diff	lwr	upr	p adj
BI-BC	-36.65625	-101.52974	28.217244	0.5478353
BR-BC	-37.56250	-102.43599	27.310994	0.5214130
BY-BC	-14.65625	-79.52974	50.217244	0.9838337
Comp-BC	-84.18750	-149.06099	-19.314006	0.0046534*
Control-BC	-93.87500	-158.74849	-29.001506	0.0012379*
BR-BI	-0.90625	-65.77974	63.967244	1.0000000
BY-BI	22.00000	-42.87349	86.873494	0.9112155
Comp-BI	-47.53125	-112.40474	17.342244	0.2651588
Control-BI	-57.21875	-122.09224	7.654744	0.1116382
BY-BR	22.90625	-41.96724	87.779744	0.8964337
Comp-BR	-46.62500	-111.49849	18.248494	0.2846792
Control-BR	-56.31250	-121.18599	8.560994	0.1219777
Comp-BY	-69.53125	-134.40474	-4.657756	0.0293720*
Control-BY	-79.21875	-144.09224	-14.345256	0.0089102*
Control-Comp	-9.68750	-74.56099	55.185994	0.9976213