Development of soil microbial communities for promoting sustainability in agriculture and a global carbon fix

David Johnson, Joe Ellington, Wesley Eaton

The goals of this research were to explore alternative agriculture management practices in both greenhouse and field trials that do not require the use of synthetic and/or inorganic nutrient amendments but instead would emulate mechanisms operating in natural ecosystems, between plant and Soil Microbial Communities (SMC), for plant nutrient acquisition and growth.

Greenhouse plant-growth trials, implementing a progression of soil conditions with increasing soil carbon (C) (C= 0.14% to 5.3%) and associated SMC population with increasing Fungal to Bacterial ratios (F:B) (from 0.04 to 3.68), promoted a) increased C partitioning into plant shoot and plant fruit partitions (m=4.41, r2=0.99), b) significant quantities of plant photosynthate, 49%-97% of Total System New C (CTSN), partitioned towards increasing soil C c) four times reduction in soil C respiration (CR) as F:B ratios increased, starting with 44% of initial treatment soil C content respired in bacterial-dominant soils (low F:B), to 11% of soil C content respired in higher fertility fungal-dominant soils (Power Regression, r2=0.90; p=0.003).

Plant growth trials in fields managed for increased soil C content and enhanced SMC population and structure (increased F:B) demonstrated: a) dry aboveground biomass production rates (g m-2) of ~1,980 g in soils initiating SMC enhancement (soil C=0.87, F:B= 0.80) with observed potentials of 8,450 g in advanced soils (soil C=7.6%, F:B=4.3) b) a 25-times increase in active soil fungal biomass and a ~7.5 times increase in F:B over a 19 month application period to enhance SMC and c) reduced soil C respiration rates, from 1.25 g C m-2 day-1 in low fertility soils (soil C= 0.6%, F:B= 0.25) with only a doubling of respiration rates to 2.5 g C m-2 day-1 in a high-fertility soil with an enhanced SMC (F:B= 4.3) and >7 times more soil C content (soil C= 7.6%).

Enhancing SMC population and F:B structure in a 4.5 year agricultural field study promoted annual average capture and storage of 10.27 metric tons soil C ha-1 year -1 while increasing soil macro-, meso- and micro-nutrient availability offering a robust, cost-effective carbon sequestration mechanism within a more productive and long-term sustainable agriculture management approach.

Title: **Soil Microbial Community Development in Agricultural Soils for Promoting Sustainability and a Global Carbon Fix.**

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Abstract: The goals of this research were to explore alternative agriculture management practices in both greenhouse and field trials that do not require the use of synthetic and/or inorganic nutrient amendments but instead would emulate mechanisms operating in natural ecosystems, between plant and Soil Microbial Communities (SMC), for plant nutrient acquisition and growth.

Greenhouse plant-growth trials were implemented in a progression of soil treatments with increasing soil carbon (C) % (C%= 0.14% to 5.3%) and associated increases in SMC population with Fungal to Bacterial ratios (F:B) ranging from 0.04 to 3.68. The flow of plant photosynthate C (g) into plant root, shoot, fruit, soil and soil respiration C partitions was quantified and demonstrated **a)** maximum total system C fixation occurs when soil C% >1.42% and F:B>1.6 and remains at this maxima in treatments with greater C% and F:B but with a

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redirection of carbon predominantly partitioned into the soil instead partitioned into plant shoot and fruit C partitions, **b)** 97% of treatment Total System New C (C_{TSN}), was partitioned into soils in treatments with low soil C% and F:B with C_{TSN} decreasing linearly (r^2= 0.94) to 48% as soil C% and F:B increase to the final treatment **c)** four times reduction in soil C respiration (C_R) (g C m^{-2} day^{-1}) as F:B ratios increased, starting with 44% of treatment initial soil C (C_{IN}) content respired in bacterial-dominant soils (F:B= 0.04), to 11% of C_{IN} content respired in higher fertility, fungal-dominant soils (F:B= 3.68) (Power Regression, r^2=0.90; p=0.003).

Plant growth in field trials, managed for increasing soil C% and enhancement of SMC population and structure (increased F:B) demonstrated: **a)** dry aboveground biomass production rates (g m^{-2} yr^{-1}) of ~1,980 g were observed in soils initiating SMC enhancement (soil C%=0.87%, F:B= 0.80) with potentials of 8,450 g in advanced soils (soil C=7.6%, F:B=4.3) **b)** a 25-times increase in active soil fungal biomass and a ~7.5 times increase in F:B over a 19 month management period and **c)** reduced soil C respiration rates, from 1.25 g C m^{-2} day^{-1} in low fertility soils (soil C%= 0.6%, F:B= 0.25) with only a doubling of respiration rates to 2.5 g C m^{-2} day^{-1} in a high-fertility soil with an enhanced SMC (F:B= 4.3) and >7 times more soil C content (soil C= 7.6%).

Applying agricultural management practices to enhance SMC population and F:B structure, in a 4.5 year agricultural field study, promoted annual average capture and storage of 10.27 metric tons soil C ha^{-1} $year^{-1}$, 20-50 times the currently observed soil carbon increase in agricultural no-till soils. These soil C% and F:B increases also promote increasing soil macro-, meso- and micro-nutrient availability offering a robust, practical and cost-effective carbon

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sequestration mechanism within a more productive and long-term sustainable agriculture management approach.

Introduction

Carbon capture and storage using agricultural land has generated worldwide interest because of the potential benefits for improving agricultural soil fertility while simultaneously addressing climate change, C mitigation and adaptation (Ohlson, Al-Kaisi & Lowery, 2014). The terrestrial biosphere contains approximately 1500 Pg C (Pg = petagram =10^{15} g = 1 billion metric tons), in the top meter of soil and there are ~800 Pg C in earth's atmosphere (Janzen, 2014). Respiration by soil microbes and other soil organisms facilitates the release of approximately 55-70 Pg C annually as CO_2, or ~7 times the emissions from anthropogenic fossil fuel consumption (~9.9 Pg C y^{-1}) (Le Quere et al., 2013). Agriculture occupies 4.9 x 10^9 hectares or approximately 37% of the world's global land area and crops are grown on approximately ~1.7 x 10^9 hectares of this "arable" land (UN FAO, 2003). Soils in natural ecosystems historically captured and held more long-residence-time carbon and previous to the year 1750, there were ~170 Pg carbon (C) stocks in agricultural soils (Paustian et al., 2000). Since then, conventional agriculture management approaches have depleted this SOC pool, contributing an extra 78±12 Pg of C into the atmosphere through breakdown and respiration of SOC into CO_2 (Lal, 2004). Effecting small adjustments to agricultural management, in a system this large towards a) improving carbon capture rates, b) increasing soil carbon retention time and c) reducing soil carbon respiration rates may offer agricultural soils as a viable path towards cost-effective and significant capture and sequestration of atmospheric CO_2 into agricultural soils.

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Efforts to restore Soil Organic Carbon (SOC) have been attempted by changing agricultural land use management practices, through conservation tillage, cover cropping, nutrient recycling of compost or manure and other sustainable practices; however, adopting these changes into conventional agricultural management methods has only demonstrated potential offsets of ~0.9±0.3 Pg C $year^{-1}$, or approximately a 10% reduction towards mitigating anthropogenic CO_2 emissions into SOC (Lal, 2004). Other scientists have concluded that conversion from plough to no-till in 67 long term field experiments captured 0.570 ± 0.140 tons C ha^{-1} yr^{-1} (West, 2002), and a study by Niggli et al. concluded that arable and permanent cropping systems of the world have the potential to capture an estimated 0.2 t C ha^{-1} yr^{-1} and pasture systems 0.1 t C ha^{-1} yr^{-1} (Niggli et al., 2009).

Natural ecosystems have traditionally outperformed conventional agro-ecosystems for carbon capture in plant biomass measured as Mean Net Primary Production (g dry above-ground biomass m^{-2} yr^{-1}). The two most productive terrestrial ecosystems, tropical rain forests and swamps and marshes, with MNPP of 2200 g and 2000 g respectively, outperform conventionally cultivated farmland, with estimated MNPP of ~650 g, by a factor of >3 times (Whittaker, 1975) without the use of conventional inorganic fertilizers. Understanding the structure and biological mechanisms of SMC in natural ecosystems for nutrient acquisition, carbon exchanges, CO_2 respiration, and plant and SMC carbon-use efficiencies will help us understand the potential negative and/or positive contribution of soil microbes to land-atmosphere exchange and terrestrial carbon cycle climate feedbacks (Bardgett, Freeman & Ostle, 2008).

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Agricultural soils offer the best scenario for C capture as they: 1) are the most impacted from the historical loss of SOC (Lal, 2004), 2) offer the best opportunity for physical manipulation of any ecosystem, 3) currently have an industrial infrastructure in place to implement alternative management strategies and 4) will benefit from increases in C as soil organic matter and its associated increases in soil fertility (Bardgett, Freeman & Ostle, 2008).

Impacts of Conventional Agricultural Practices on Soils Microbial Communities

Many of the current conventional agricultural management practices have proven detrimental towards the sustainability of the world's agro-ecosystems and to their capacity to store C. Conventionally managed agricultural fields are eroding through the native stock of topsoil at an average of 1–2 orders of magnitude greater rates of soil loss than those of soil production thus limiting the lifespan of our agricultural system (Montgomery, 2007). An 86 year long-term bare-fallow study of the impacts of fertilizers and amendments on soil physical properties concluded these amendments degraded soil aggregation, increased bulk density (compaction) and lead to strong acidification of soils (Paradelo, Oort & Chenu, 2013).

Conventional methods for cultivation of soils, besides affecting soil chemistry and structure, reduce biological activity due to the reduction of macro-aggregates which provides an important micro-habitat for microbial activity (Dick, 1992). Wide usage of inorganic or synthetic fertilizers in conventional agriculture have negative impacts on biodiversity at various levels including plant, vertebrate and non-vertebrate groups (McLaughlin & Mineau, 1995). Conventional agricultural management employs practices (bare fallows, synthetic fertilizers,

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pesticides, lack of green fallows or manure applications) that decrease soil fertility through: reduction of soil C and microbially-originated C stocks, escalation in soil C decomposition rates and disruption of microbial food webs (Huber et al., 2008; Horrigan, Lawrence & Walker, 2002; Kuzykov, 2010). Conventional agricultural practices reduce soil fungal populations through reduced incorporation of fresh plant C and physical disturbances to soil structure (Dighton, 2003), damaging associated SMC that previously supported plant nutrient acquisition, plant pathogen resistance (Doornbos, van Loon & Bakker, 2012) and beneficial plant/SMC interactions (Penton et al., 2014).

Addition of conventional fertilizers (nitrogen and phosphorus) as nutrient amendments has the unintended consequence of diminishing soil C and reducing beneficial associations between plants and SMC (Fliessbach et al., 2000). Cropping practices and the use of nitrogen fertilizers are estimated to cause 78% of the total soil N_2O emissions in the United States (EPA, 2007), promoting emissions having a greenhouse warming potential 268 times that of CO_2 (Myhre et al., 2013). Application of nitrogen fertilizers were first thought to benefit agricultural soils and perceived to sequester SOM by increasing input of crop residues. A century of SOM data, at one the world's oldest experimental site under continuous corn production (Morrow Plots, University of Illinois), concluded that synthetic N fertilization exceeded grain N removal by 60-190% with a net decline in soil C despite increasingly massive residue C incorporation (Khan et al., 2007). In another study, synthetic N fertilization demonstrated negative effects on soil organic N content, shifting native organic N towards increased mineralization along with a further shifting of soil organic matter (SOM) to be mineralized (respired) as CO_2 (Mulvaney,

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Khan & Ellsworth, 2009). Similarly, applications of phosphorous fertilizer suppresses the formation of plant's associations with mycorrhizal fungi, curbing activity of the group of microbes (*Glomeraceae*) that contribute to the formation of glomalin, a structural component in the cell walls of fungal hyphae, representing up to 1/3 of the world's native soil carbon resources (Wright & Nichols, 2002).

Associations and Interactions of Soil Microbial Communities

Microbes live, for the most part, in biological colonies, co-existing with other bacterial species and implementing self-coordinated bio-communication processes designed to promote mutual, neutral and manipulative symbioses (Surette & Keller, 2006). Bio-communication may occur between bacteria, fungi and host organisms with production, release, uptake and interpretation of signal molecules (Witzany, 2011). As microbial populations increase, production and release of these chemical signal molecules (auto-inducers) is stimulated, enabling cell-to-cell communication of microorganisms, allowing coordination of activities and functioning of many individual cells as a single multicellular system through quorum sensing (Miller & Bassler, 2001). Quorum sensing initiates functional and coordinated gene expression (symbiosis, virulence, competence, conjugation, antibiotic production, motility, sporulation and biofilm formation) strongly correlated to the population density of participating microbes (Miller & Bassler, 2001). This cell-to-cell communication occurs both within and between bacterial species and there is mounting evidence suggesting specific responses are coordinated with host organisms as well (Miller & Bassler, 2001).

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Plants, acting as host organisms, often secrete 30% to 60% of the C captured as plant photosynthates (energy and nutrient resources) to support mutualistic relationships with SMC (fungi and bacteria) (Buck, 2004). Correspondingly, mycorrhizal fungi have also been implicated in secretion of signaling molecules and nutrients for soil bacteria triggering the degradation of substrates for acquisition of specific nutrients that are then made available for transport and assimilation by both the mycorrhizal fungi and their plant host (Bonfante, Visick & Ohkuma, 2010). The biodiversity of SMC is also believed to play fundamental roles in nutrient release, nutrient formation, soil structure maintenance and contribute to water storage and transfer in soils (Lavelle, 2000).

Soil Carbon Stability

SOM does not consist of pools of biochemically complex and structurally uniform molecules and many notions of molecular recalcitrance as a method of understanding SOM stability as long mean residence time (LMRT) soil C is losing support in the literature (Schmidt et al., 2011; Kleber et al., 2011). Organic matter remains in the soil because of physico-chemical and biological influences from the surrounding environment reducing the rate of decomposition and enabling organic matter to persist and build up in soils (Schmidt et al., 2011). Young SOM does not differ greatly from older SOM in structure and complexity and its composition does not indicate that humification processes are creating chemically recalcitrant humic substances with complex aromatic structures, like lignified or humified soil organic carbon moieties (Schmidt et al., 2011). New findings support microbial activity as the primary active agent for SOM stabilization, and it is most likely that constituent C, after integration into

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166 new microbially derived molecules, remains in the soil longer (Chabbi & Rumpel, 2009). Soil
167 microbial biomass, present as bacterial and fungal cell wall structural components and organo-
168 mineral complexes, resulting from microbial alteration, has been identified as a potential
169 source of LMRT SOM (Kleber et al., 2011; Miltner et al., 2011).

170 Soil microbial communities influence the production of LMRT soil C through: **a)** physical
171 protection by adsorption onto minerals as organo-mineral complexes with LMRT C lasting for
172 centuries (Dungait et al., 2012), **b)** soil structure aggregation through secretion of microbial
173 glues and mucilages (Sollins et al., 2009), **c)** fungal structural glycoproteins (glomalin) in binding
174 soil particles with fungal hyphae, potentially representing ~30% of worldwide soil carbon with
175 ~40 year lifespans (Wright & Nichols, 2002), and **d)** spatial inaccessibility through intercalation,
176 hydrophobicity and encapsulation (Lutzow et al., 2006).

177 A recent study of C mean residence times (MRT) in soils, studying four surface soils with
178 a wide range of mineralogy, climates and vegetation types attributed C MRT up to ~985 years
179 to a layered model of organic matter accumulation (Sollins et al., 2009). The innermost layers of
180 these organo-mineral surfaces were protein-rich and accompanied by an increase in the
181 "microbial signature" of the organic matter (decrease in C/N ratios, decrease in lignin content
182 accompanied by an increase in degree of lignin oxidation and an increase in $^{\delta 13}C$ and $^{\delta 15}N$)
183 associated with a corresponding decrease in vascular plant tissue signatures (Sollins et al.,
184 2009). Soil microbes, fungi in particular, assist in the formation of persistent SOM through
185 stabilization of SOC with the formation of micro-aggregates, production of hydrophobins, and
186 chaplins (King, 2011). The relative abundance of fungi to bacteria (high fungal:bacterial

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187 biomass ratios) in soils may determine the stability of the carbon formed in soils (Six et al.,
188 2006).

189 **Soil Carbon Respiration**

190 The population of SMC and their activity increases with the accumulation of soil C (Shi,
191 Bowman & Rufty, 2012). Soil microbes have been implicated in respiration of SOC with multiple
192 studies on the effects of temperature, moisture, oxygen, pH, nutrients and SOC quantity
193 (Janzen, 2014) but few studies have considered the impact of microbes for recycling plant
194 derived carbon towards more stable microbial-signature soil carbon compounds and their
195 potential influence on soil respiration rates and for increasing biomass production.

196 Soil respiration is a complex flux combining C flow from root-derived respiration
197 (autotrophic) and microbial decomposition of organic matter (heterotrophs) within a plant/soil
198 matrix (Heinemeyer et al., 2011). Heterotrophic bacteria follow two paths in the transformation
199 of soil organic matter: **a)** they produce new bacterial biomass and **b)** they mineralize organic
200 carbon into inorganic carbon (CO_2) through respiration processes.

201 Bacterial growth efficiencies represent the amount of bacterial biomass (secondary
202 production) that is produced per unit of organic C substrate assimilated relative to the balance
203 of CO_2 respired. Estimates of bacterial growth efficiencies range from <5% to as high as 80%,
204 and there is little understanding of the ecological or physiological mechanisms that regulate this
205 enormous range (del Giorgio & Cole, 1998). Under poor growing conditions, of high C:N ratios
206 and low C accessibility, bacterial growth efficiencies range between 5% to 20%, where in

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nutrient rich systems bacterial growth efficiencies can range between 40% and 80% (Taylor & Townsend, 2010). Similarly, fungal growth efficiencies (FGE) range from 5% to 77%, and again these ratios appear to be similarly dependent on nutrient availability (Six et al., 2006). Both of these studies indicate high organic C substrate-to-microbial biomass conversion efficiencies are obtainable for bacterial and fungal communities when grown in nutrient rich conditions. Recent studies on nutrient rich and nutrient poor forests have observed carbon use efficiencies 5 times higher in nutrient rich forests, when compared to forests in nutrient poor soils, and demonstrated substantial increases in carbon uptake and carbon sequestration capability in nutrient rich forests (Fernandez-Martinez et al., 2014).

Priming effects (PE) are phenomena that soil microbes exhibit for decomposing older soil organic matter using fresh carbon as a source of energy mobilizing C reserves assumed to be protected from microbial attack (Fontaine et al., 2007a). Fungi are now suspected in regulation of PE through mediation of long term sequestration of carbon and nitrogen in soil using a “bank” mechanism for regulating nutrients and carbon in soils (Fontaine et al., 2007b). When nutrient availability is high, PE is low, reducing decomposition pathways and allowing sequestration of nutrients and carbon. Correspondingly, when nutrient availability is low, microbes extract necessary nutrients from SOM reducing the amount of C in those soils. This banking mechanism is suspected to promote synchronization of available soluble nutrients to plant requirements contributing to long-term SOM accumulation in ecosystems (Fontaine et al., 2007b).

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Interactions between plants and SMC are an integral part of our terrestrial ecosystem and optimization of plant and microbe relationships will be necessary to promote improved plant growth and more efficient carbon sequestration mechanisms (Wu et al., 2009). Research in this manuscript explored the influence of increasing SMC population and F:B structure on plant growth and for increases in soil carbon content using easily-adoptable agronomic practices that are practical for farmers (commercial to third world) to adopt into their agricultural enterprise.

Understanding the influence of SMC in agro-ecosystems requires accurate C flux modeling and will require soil C and N dynamics to include below-ground carbon and nitrogen flux, the partitioning of root and mycorrhizal exudates, and the effects these components have for C sequestration in soils by microbial communities (Chapin et al., 2009). To these ends: This research pursues the hypotheses that:

1) *SMC have considerable impact on the flow of C and N within the plant/SMC system and these flows are dependent upon the initial soil C content and soil fertility as determined by SMC population and F:B structure,*

2) *Changing the focus of agricultural management practices, to promote development of SMC population and structural diversity (F:B) will promote and support development of mutualisms between plants and SMC and*

3) *These mutualisms will produce a carbon sequestration platform that will economically capture and more efficiently store large quantities of atmospheric CO_2*

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as microbial-signature soil C while reducing soil respiration within a more sustainable agricultural system.

Materials and Methods

Greenhouse Experiments

Greenhouse experiments were designed to explore growth characteristics of chile plants (*Capsicum annuum*, variety Big Jim "Heritage") in six soil treatment conditions mixed for soil C% and F:B. **(Table 1).** Treatment soils in this research were formulated by mixing two soil components consisting of: **1)** a fungal dominant soil, a compost with a homogenous microbial community structure obtained from a Johnson-Su composting bioreactor (Johnson & Su, 2010) **(S1-Figure 1)**, and **2)** a bacterial dominant soil, an alluvium (southwestern desert sand/clay mixture) obtained from a local desert arroyo **(S1-Figure 2)**. The six greenhouse experimental treatment mixtures (0, 1, 2, 3, 4, 5) were based on mixtures of these two soils (dry mass ratios) designed to demonstrate linearly increasing levels of C (g), N (g), and an associated soil microbial community metric, Fungal to Bacterial Ratio (F:B). Soil C, N and F:B ranged between: C% (0.14% - 5.3%C), N% (0.0001% – 0.004%) and Total F:B ratios (0.04 -3.68) **(Table 1)** for the 6 treatment soils**.**

Inorganic Soil Nutrient and Soil Microbial Community Analyses

Inorganic soil nutrient analyses, for both the greenhouse and field trial soils, in this research, were performed by Soil, Water and Forage Analytical Laboratory, Oklahoma State University for soil nutrient profiles of TC, TN [%]; and P, K, Mg, Mn, Fe, Cu, Zn [mg/kg]. Soil

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microbial community analyses were sent to Soil Foodweb Oregon LLC, 635 SW Western Blvd, Corvallis, OR, 97333 to enumerate fungal, bacterial, protozoan and nematode populations with sample preparation, staining procedures and biomass quantification using direct microscopy (Stamatiadis, Doran & Ingham, 1990; Ingham, 1995; Ekelund, 1998). Laboratory results for the biological components of the compost and alluvium soil used in this study are in the supplementary material **(S1-Figure 1, S1-Figure 2**).

Loss on Ignition Analysis

Carbon mass (g) for each treatment was derived from calculations for proportions of the dry mass of each of the two soil components being mixed and then translated to these soil and their original moisture content; therefore, beginning soil C% for each treatment mixture was confirmed using follow-up Loss-on-Ignition (LOI) soil analyses (SOC%) from triplicate samples of treatment soil mixtures (0-5). Soil samples from each treatment (6-10 g) were pre-weighed, dried overnight 105^{o}C in a muffle furnace, weighed again for dry biomass and then subjected to a follow up soil organic carbon oxidation process for 2 hours at 375 ^{o}C with a final weighing and calculation of LOI.

Greenhouse Cultivation Procedures

Chile plant seeds (*Capsicum annuum*, Big Jim "Heritage" variety) were planted in each of the six treatments (0, 1, 2, 3, 4 and 5), (n=5) and allowed to grow for an 86-day growth period. Four seeds were planted in each plant container and then thinned to 2 healthy plants per container approximately 10 days after germination. Plant containers were watered daily with

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287 approximately 50-75 mL of distilled water. Photosynthetically active radiation (PAR) was
288 supplied by two (2) 2' x 4' SlimStar, 6 bulb, high-output T-5 grow lamps with 30,000
289 lumens/fixture (6,400 K spectrum Grow bulbs) operating for 12 hours per day for the 86 day
290 growing period. After the growing period, root biomass was removed from the soils in each
291 treatment, the soils were weighed and then subsamples from each of the treatment potting
292 containers were pooled according to the six treatments (0, 1, 2, 3, 4 and 5) and then shipped to
293 Oklahoma State University Soils and Water Testing Laboratory to be analyzed in triplicates, for,
294 TC, TN (%), P, K, Ca, Mg, Mn, Fe, Cu, Zn, EC (mg kg^{-1}), pH and moisture content (%). Plant tissue
295 (roots, canopy and chile) were harvested separately, oven-dried for 3 days at 45^{o}C in pre-
296 weighed oven-dried paper bags and re-weighed to the closest 0.0001 g on a Mettler AE200
297 balance.

298 **Field Experiments**

299 A Biologically Enhanced Agricultural Management (BEAM) approach, designed to
300 optimize the growth and diversity of SMC was employed in the BEAM field plots in this
301 research. The BEAM process uses no synthetic fertilizers, traditional quantities of water,
302 standard agricultural techniques and no specialized equipment. SMC development in BEAM
303 field plots was promoted using inoculation with minimal applications (~.25 ton /acre; ~60%
304 moisture content) of a fungal-dominant compost produced in a Johnson-Su composting
305 bioreactor (Johnson & Su, 2010). Continuous growth of both cover and/or commodity crops is a
306 key element of the BEAM process for production of plant biomass and plant exudates to

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encourage development of soil carbon, plant biomass and plant exudates to support SMC development.

All experimental field studies were conducted on New Mexico State University agricultural plots located at coordinates: 32º 11' 37.60" N; 106º 44' 21.68" W. Soil types at the field site were composed of Armijo (Fine, smectic, thermic, Chromic Haplotorrerts) and Harkey (coarse-silty, mixed, superactive, calcareous, thermic typic Torrifluvents) clay loams (USDA NRCS, 2014). No fertilizers were used on any of the field sites employing BEAM and standard amounts of irrigation water were applied (Seasonal, 3- 4 Ac feet). No pesticides were used during the plant growth period and no moldboard plowing or deep soil-turning procedures were used on the BEAM plots.

Cover crops for field studies consisted of multi-species winter covers (Bell Beans, Biomaster Peas, Dunsdale Peas, Common Vetch, Cayuse Oats, Common Vetch, Purple Vetch) and single species summer covers including Colorado River Hemp (*Sesbania exaltata*), a summer annual legume native to the U.S. southwest and Arugula "Wild Rocket" (*Eruca sativa*) used for short-season (less than 35 days) intercropping for nitrogen scavenging and heavier root penetration in soils. All BEAM fields were planted with the appropriate seasonal crop described above, allowed to grow to blossom stage, green chopped with a flail mower, "lightly" disked (top 4 inches) into the soil and then quickly replanted to start the process again.

Field trials were conducted on adjacent agricultural soil plots consisting of 1) a field plot with a beginning soil C= 0.43%, and an ending soil carbon= 1.52%C managed over a 4.5 year

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period (BEAM-4.5) 2) an improved BEAM plot with soil carbon=7.9% soil C (BEAM-7.9%C), 3) a conventionally managed treatment plot (Conv) incorporating: synthetic fertilizers, deep ripping, plowing, insecticides, herbicides, bare fallows and traditional cropping with cotton and 4) a control plot (Control) same management as Conv but with no fertilizers.

Treatment metrics were taken on the BEAM-4.5 treatment for: plant biomass, standard inorganic soil nutrient profiles N, P, K, Mg, Mn, Zn, Cu, Fe (mg/kg), soil C (%) and N (%) and F:B ratios using the procedures and laboratories listed above in greenhouse experiments. MNPP, (as g dry aboveground biomass m^{-2}) for BEAM-4.5 and BEAM-7.9%C was estimated through random selection and harvesting of biomass from multiple "representative" field samples. These samples were averaged, analyzed for wet and dry plant biomass and then extrapolated to hectare areas.

Field Soil Sampling Procedures

Field soil samples were conducted by choosing twenty five random locations and drilling 5/8" soil cores approximately 10-12 inches deep, for each soil sample. Cores were mixed until homogenous and placed into 1 quart double sealing plastic bags, labeled, pre-cooled and shipped within 24 hours to the corresponding labs for analysis of standard soil fertility and soil biological foodweb characteristics.

Soil Respiration

Reliable methodologies for insuring accurate measurement of soil CO_2 efflux are still under debate and development (Pumpanen et al., 2004; Kuzykov, 2010) therefore an

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347 inexpensive and simple static alkali trap methodology was chosen to measure soil respiration.
348 Alkali traps can yield overestimates of low fluxes and underestimates of high fluxes but can be
349 reliably calibrated for intermediate ranges of CO_2 flux (Davidson et al., 2002). Accurate soil
350 respiration measurements can be affected when insertion of sampling collars sever soil root
351 structures, when coupled with a preferential practice of taking only daytime measurements
352 (Heinemeyer et al., 2011) and when the surface area of the alkali reaction vessel is less than 6%
353 of the soil surface area sampled (Raich & Nedelhoffer, 1989). The parameters for the use of
354 static alkali reactors in this research avoided these drawbacks following methodological
355 guidelines to insure accurate soil respiration measurements. While not an absolute quantitative
356 assessment, the static alkali reactors systems were able to render a reliable, internally-
357 comparable analysis of CO_2 emissions, as well as soil respiration values (g C m^{-2} day^{-1}) within the
358 historically observed ranges when compared to different ecosystems and types of vegetation
359 (Raich & Schlesinger, 1992).

360 Soil C respiration C_R (g), for each of the greenhouse and field treatments and trials, was
361 measured with static alkali reactors placing a 50 ml plastic centrifuge tube containing 15 mL of
362 1M KOH, with a cross-sectional area of ~25% of soil surface sampled, placed ~3 cm into the soil.
363 Reactors were covered with a ~1 liter jar inserted 2 cm into the soil, and allowed to remain
364 undisturbed for a 24 hour period. The 50 ml tubes were then removed from the reactors,
365 capped and taken to a laboratory for titration analyses.

366 Prior to titration, the 1 M HCl titrant concentration was confirmed with comparison to a
367 1 M KOH control and adjusted to exhibit a 1 M HCl concentration relative to an unexposed

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368 control reactor. The exposed static alkali reactors were treated with 1 mL aqueous solution of
369 saturated BaCl and 1 drop of the pH indicator (phenolphthalein) and then titrated with the
370 standardized 1 M HCl to an endpoint where a color change (from pink to clear) occurred
371 (process was also monitored with an Acumet benchtop pH meter). The volume of titrant was
372 then used to calculate the amount of CO_2 absorbed in the static alkali reactor solution relative
373 to the area of the opening of the mouth of the 1 liter bottle used to place over the alkali reactor
374 tube. Preliminary sensitivity analyses **(S1-Figure 3)** (with 1, 2 and 3 day reactor operating times)
375 were conducted to confirm CO_2 absorption characteristics, variance and reproducibility and
376 determine best practices for estimating respiration of treatment soils.

377 Soil respiration measurements were conducted on each of the greenhouse treatments
378 (0-5) at selected intervals (4 separate samplings, 38, 46 ,58 and 86 day) using a non-repeating
379 and randomly chosen plant container over the 86 day growth period. Soil C respiration C_R (g)
380 values were based on a cumulative analysis for each of the previous number of daily time
381 periods and for each of the sampling intervals. Respiration rates were adjusted for static alkali
382 reactor soil surface area then totaled for the 86 day growing period.

383 Respiration rates of 4 field plots and a desert soil were conducted once a week (every
384 two weeks in the winter) for approximately one year (32 sampling periods) with static alkali
385 reactors using the same procedure described above to correlate relative soil CO_2 emissions (g
386 m^{-2} day^{-1}). These treatments consisted of **1)** BEAM-4.5 a plot initializing BEAM in an agricultural
387 field implementing BEAM for 4.5 years with a soil C= 1.52 %, **2)** a conventionally managed plot
388 (Conv) with soil C= 0.6%, **3)** a Control plot with no fertilizer applications (Control) with soil C=

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0.6%, **4)** a Desert treatment (Desert) with soil C= 0.3% and **5)** an advanced BEAM plot with soil C= 7.9% (BEAM-7.9%C) **(S1-Figure 4).**

Results:

Greenhouse Experiments

Greenhouse experiments assessed dry plant biomass (g), system partitions of C (g C), system partitions of nitrogen (g N) and treatment respiration (g C) measurements of chile plants in six soil treatments (Treatments 0,1,3,4 and 5 (n=4) and Treatment 4 (n=3) of linearly increasing beginning soil C%, N% and F:B ratio (on average 1 reactor in each treatment was excluded due to inadequate plant germination, insect damage and/or soil loss events that would lead to inaccurate metrics for mass balance) **(S3-Table 5)**. Loss-on-ignition analysis was conducted on all 6 treatments (0-5) to assess validity of mixing protocol and beginning-treatment soil C% using dry weight of the two component mix (compost/alluvial sand). Results from a GLM regression analysis, comparing initial calculated treatment soil C% with LOI analyses, produced a linear trend line with r^2=0.98 (P=0.0002) **(S1-Figure 3).**

Carbon

System C mass (g) flow was evaluated in seven partitions: root C (C_{RT}), plant shoot C (C_{SH}), plant fruit C (C_{FR}), initial soil C (C_{IN}), new soil C (C_{NS}), soil respiration C (C_R), and total system new C (C_{TSN}) a summation of all new and/or replacement (respiration) carbon in the above partitions. Aboveground C partition mass (C_{SH} and C_{FR}) exhibited positive linear regression trends, (r^2=0.96, 0.96 respectively), when compared to soil F:B **(S1-Fig. 6**)

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(comparison of C partitions to F:B and/or C_{IN} are synonymous as treatment mixtures were designed to yield linear increases in F:B and C_{IN} and a linear regression analysis of these two yields an r^2>0.99) Belowground C partitions, (C_{RT}, C_{NS} and C_R) deviated from the linear regression analysis exhibiting 2nd order polynomial regression correlations with r^2 =0.97, 0.84, and 0.97 respectively **(S1-Fig. 6**). There was an increase in partitioning of C_{TSN} into the C_{NS} partition up to Treatment 2 (1.42% C and F:B= 1.60), after which the rate of increase slowed and reversed but was still significant on to the final treatment **(S1-Fig. 6)**.

A further analysis of carbon partitioning, comparing Aboveground C mass (g) partitions (C_{SH} + C_{FR}) with Belowground C mass (g) partitions (C_{NS} + C_R) in each of the six treatments was conducted. A derivative of the belowground C partitions, estimated as a percent (%) of C_{TSN} partitioned into the soil as (C_{NS} + C_R)/ C_{TSN} , yielded "%C_{TSN} Diverted to Soil", with the balance of C mass partitioned in the Aboveground C partitions (C_{SH} + C_{FR}) (**Figure 1**). The C_{RT} partition, represented less than 14% of C_{TSN}, was deleted from the Belowground C in this analysis to isolate and identify only C resources (plant exudates) directed into the soil structure. Treatment "0" demonstrated a 97% flow of "%C_{TSN} Diverted to Soil", into the Belowground C partition allowing only 3% partitioned to Aboveground C. As the Treatment C_{IN} increased, along with its associated SMC population and F:B ratio **(Table 1). T**he "%C_{TSN} Diverted to Soil", parameter decreased linearly (r^2=0.94) to an end point of the six treatments where 49% of C_{TSN} flowed into the soil and the balance, less C_{RT}, was diverted to the Aboveground C partitions, (C_{SH} and C_{FR}) (**Figure 1**). Statistics for carbon partitions mass, C% and plant component C% are in **S2-Tables 1,2,3,5,6,7 and 8**.

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Nitrogen

System N mass (g) flow was evaluated in each of six partitions: root N (N_{RT}), plant shoot N (N_{SH}), plant fruit N (N_{FR}), initial soil N (N_{IN}), new soil N (N_{NS}) and total system new N (N_{TSN}) a summation of all new system N in the above partitions. Aboveground N mass (g) (N_{SH} + N_{FR}) exhibited positive linear regression trends when compared to treatment initial soil F:B, (r^2=0.99, 0.96 respectively) (**S1-Figure 7**). Belowground N mass partitions, (N_{RT} and N_{NS}) deviated from a linear correlation, exhibiting 2nd order polynomial regression correlations with r^2's equaling 0.97 and 0.84 respectively (**S1-Figure 7**). The N_{NS} partition followed trends similar to those observed in the soil C mass assessment (**Figure 1**), increasing up to Treatment 2 (1.42% C and F:B = 1.60) to an apex and then decreased to no N being input into the soil in the final Treatment 5 **(S1-Figure 7).**

Analyses were conducted in each of the six treatments, comparing N partitioning of a derivative of the N_{TSN} and N_{NS} partitions (N_{NS}/N_{TSN}) denoted as, "%N_{TSN} Diverted to Soil", portraying the percent of total N_{TSN} directed into the soil structure with the balance into the Total Plant N (N_{RT} + N_{SH} + N_{FR}) (**Figure 2**). The "0" treatment, un-amended with compost, demonstrated a 36% flow of N_{TSN} into the soil structure. As treatment C_{IN} increases, along with its associated linear increases in SMC population and increasing F:B and the first introduction of SMC inocula from the compost amendment, the "%N_{TSN} Diverted to Soil", increased to 86% and then decreased from that treatment to the final treatment in a second-order curvilinear regression trend line (r^2= 0.99) to a zero point in Treatment 5 (3.68% C, F:B= 5.3) with no new N

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(g) was flowing into N_{NS} **(Figure 2).** Statistics for Nitrogen partitions mass, N% and plant component N% are in **S2-Tables 1,2,4,5,6,7 and 8**.

Soil CO_2 respiration rates along with total respired C in the 6 greenhouse treatments over the 86 day growing period (g C m^{-2}), were compared with C_{IN} to derive a "Percent of C_{IN} Respired". The evaluation depicted in **Figure 6**, comparing C_R to C_{IN} demonstrates how C_R rates decrease from ~44% respiration of available soil C" (g) in "Treatment 0" to ~11% of C_{IN} respired in "Treatment 5" as associated treatment SMC and F:B ratios increase. The change in C_R over the treatment C_{IN} range represented a 4 times reduction in % of soil C_{IN} respired relative to a 38 times increase in soil C, and was best represented with a negative exponential power regression correlation to the Treatment C_{IN} values with an r^2=0.87 **(Figure 6**).

Field Experiments

Application of the BEAM approach, in the BEAM-4.5 field treatment, tracking carbon content in MNPP (g dry above ground biomass m^{-2} $year^{-1}$), soil macro-, meso- and micro-nutrients and increases in soil C% was conducted to assess soil fertility improvement and the impact of BEAM for improving C capture in agricultural soils.

MNPP during this 4.5 year period in BEAM-4.5 totaled approximately 40.26 mt MNPP ha^{-1} grown and returned to the soil **(Table 2).** Beginning soil C% on this field was 0.43% in a soil with a bulk density of 1.45 g/ml. After 4.5 years, the soil C% was 1.52% demonstrating an increase of 1.09% C, or a 48.17 mt C ha^{-1} soil C increase (Table 2).

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469 The MNPP biomass growth rates during a five month off-season growing period from
470 11/8/2011 to 4/8/2012, were compared in two BEAM soils, **1)** BEAM-4.5, a field experiencing
471 4.5 years of BEAM with an original starting soil C% of 0.43% and a current soil C% of 1.52% to **2)**
472 an improved BEAM field trial with 7.9% soil C (BEAM-7.9%C). The BEAM-4.5 soil produced ~968
473 g m^{-2} MNPP (4.36 mt C ha^{-1}) and the BEAM 7.9%C produced 4,736 g m^{-2} MNPP (21.31 mt C ha^{-1})
474 within the 150 day growing period **(Figure 3).**

475 **Soil macro and micro-nutrient characteristics** were measured for a 19 month period at
476 the beginning of implementation on the BEAM-4.5 treatment to determine the effect of
477 intensive application of cover crops on soil macro-, meso- and micro-nutrient concentrations.
478 The 19 month period included the planting, maturation, green-chopping and disking of 3
479 successive cover crops **a)** Sesbania (summer 2010), **b)** Mixed Winter Cover (Winter 2010/2011),
480 **c)** Sesbania (Summer 2011). Soil samples were taken over five sampling periods, at the
481 beginning and at months 6, 8, 15 and 19, and analyzed for N (Kjeldahl), P, K, Ca, Cu, Fe, Zn, Mg,
482 Mn (mg/kg), and Soil Organic Carbon (SOC). Results from the 19 month study with 5 sampling
483 periods indicate all macro-, meso- and micro-nutrients increased accordingly: (N) ~64.5%, (P)
484 ~63.7%, (K) ~36.7%, (Ca) ~75.8%, (Cu) ~40.2%, (Fe) ~1110.4%, (Zn) ~62.0%, (Mg) ~82.6%, (Mn)
485 ~1135.1% and (SOC) ~88.0%. Elemental percent increase, regression trend lines characteristics
486 and r^2 for eleven nutrients are in **(Table 3).**

487 **SMC fungal and bacterial biomass and ratio** analyses were conducted on three adjacent
488 field plots comparing a soil experiencing BEAM for one and one half years (BEAM-4.5), a control
489 experimental plot with 5 previous consecutive years with no application of synthetic fertilizers

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or amendments (Control) and an experimental plot experiencing 5 consecutive years of conventional agricultural management [*two successive cotton crops and one bare fallow during this analysis*] (Conv). Active fungal populations (µg g^{-1} dry soil) after one year's application of BEAM on BEAM-4.5 were 24-25 times higher **(Figure 4)** when compared to the control (Control) and the conventionally managed soil (Conv)**.** One and one half years after the initiation of BEAM on BEAM-4.5 the active fungal to active bacterial ratios (F:B) in BEAM soils were 4.4 and 3.0 times higher than the control (Control) and the conventionally managed soil (Conv) respectively **(Figure 5).**

Soil respiration results for one year's sampling (32 sampling periods) of 5 field test plots: **a)** BEAM-7.9%C, **b)** (BEAM-4.5, **c)** Conv, **d)** Control and **e)** a desert soil are represented by a candlestick graph in **(Figure 7).** The upper and lower bounds of the vertical line in this figure represent the maximum and minimum values recorded for results in each of the five treatments. The rectangular boxes represent values recorded for each treatment between the 1^{st} and 5^{th} quintile, representing 60% of the recorded soil respiration measurements for a one year sampling period. The BEAM-4.5 (soil C= 1.52%) treatment exhibited a mean respiration value of ~1.4 g C m^{-2} day^{-1}, a conventionally managed plot (Conv) with soil C%= 0.6% exhibited a mean respiration value of ~1 g C m^{-2} day^{-1}, the control plot (Control) with a soil C%= 0.6% exhibited a mean respiration value of ~1.1 g C m^{-2} day^{-1}. The advanced BEAM soil treatment (BEAM-7.9%C) exhibited a mean respiration value of ~4.4 g C m^{-2} day^{-1}. A Desert treatment (Desert) with a soil C%= 0.3% was used to represent a natural-environment control, and this soil exhibited mean respiration values of ~1 g C m^{-2} day^{-1} (**Figure 7**).

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Discussion:

All above ground C and N (g) partitions (C_{SH}, C_{FR}, N_{SH} and N_{FR}) demonstrated strong linear correlations (r^2= 0.96, 0.96, 0.99, 0.96 respectively) potentially supporting soil C_{IN} as an energy and nutrient resource. The observed linearity in the increase of aboveground biomass (g) relative to C_{IN} (g) in this greenhouse experiment could be explained from a nutrient-resource-availability perspective where the increasing concentrations of soil C with its associated nutrient content promote corresponding plant growth.

The C and N partitions that do not follow a resource-availability hypothesis (C_{RT}, C_{NS}, C_R, N_{RT} and N_{NS}) are best correlated with curvilinear 2nd order polynomial regression analyses indicating other mechanisms are at work. When considering the percentage of C_{TSN} flowing into the soil partitions an interesting linearity returns with the "%C_{TSN} Diverted to Soil", following a negative linear trend (r^2=0.94) with 97% of "%C_{TSN} Diverted to Soil", in Treatment 0 reducing to 48% of "%C_{TSN} Diverted to Soil", in Treatment 5 **(Figure 2).** The results from these experiments reveal the ability of the plant/SMC ecosystem to preferentially partition up to 98% of captured C into the soil environment and even in fertile soils still dedicate >48% of photosynthate towards supporting SMC. At first consideration, the reallocation of photosynthetic C towards development of soil C, soil N and SMC development would appear detrimental to the plant's survival but it may offer other benefits for immediate and/or future soil development promoting plant growth.

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Nitrogen partitioning into "%N_{TSN} Diverted to Soil", followed a different trend as treatment C_{IN} and F:B increased with the initial "Treatment 0" (C_{IN}=0.14, F:B= 0.04) demonstrating an initial 36% of the flow of "%N_{TSN} Diverted to Soil". "Treatment 1" (C_{IN}=0.71, F:B= 0.84) was the first treatment with the addition of SMC inocula and the "%N_{TSN} Diverted to Soil", increased to 86% N_{NS} partition. The partitioning of N_{NS}, after Treatment 1, continued declining in a negative curvilinear regression trend line mode (r^2=0.99) from "Treatment 1" to an end point in "Treatment 5" (C_{IN}= 5.3%, F:B= 3.68) where none of N_{TSN} was diverted to soil. The SMC influence on partitioning of "%N_{TSN} Diverted to Soil", indicates the potential actions of plant/SMC signaling mechanisms in a control response to C_{IN} and/or N_{IN} concentrations (surplus of deficit) in each treatment.

There have been many field observations indicating soil function is less productive when soil C percentages drop below 1.7% (<3% SOM) (Loveland & Webb, 2003) but there has been a lack of experimental evidence to validate these observations. The results of this experiment appear to support observations of this threshold where: **a)** the maxima of C_{TSN} production is reached by Treatment 2 (C%= 1.4, F:B= 1.6) and from that point on C_{TSN} maintains this maxima but it is increasingly partitioned into C_{SH} and C_{FR} and with less C partitioned into C_{NS}, **b)** the zenith of both the trend lines of C_{NS} and N_{NS} peak at ~1.7%C (at the apex of both C_{NS} and N_{NS} trend lines or a little after Treatment 2, (soil C%= 1.4%, F:B= 1.6) where there appears to be a satiation point when partitioning of both C and N into the soil environment begins to decrease. This decrease in C and N flow into C_{NS} and N_{NS} is correlated with an accompanying redirection of the flow of C and N resources into C_{SH}, C_{FR}, C_{RT}, N_{SH}, N_{FR}, N_{RT}, **(Figure 1 & Figure 2**) potentially

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explaining the field observations in Loveland & Webb. Soil N, in the greenhouse trials, is most likely increased through the interaction between chile plants and "free-living" nitrogen-fixing soil bacteria (eg., *Azotobacter*, *Clostridium, Anabaena*) since chile plants are not observed to form nitrogen-fixing nodules. Soil N increased up to the ~1.7% C_{IN} (after Treatment 2, F:B= 1.6) with a marked decline after that point **(Figure 2)** indicating there was sufficient soil N at that point in the plant/SMC ecosystem to allow the plant to partition more of its photosynthates into C_{SH} and C_{FR}. Based on the observations in this study, the plant/SMC ecosystem, as a "collaborative entity", appears to be capable of preferentially directing the flow of energy and nutrient components towards improving either or both of the plant and/or soil C and N partitions.

Soil C Respiration

The observed reductions in C_R, in the greenhouse portion of this research, demonstrated a 4-fold reduction in soil CO_2 emission rates as C_{IN} (g) and SMC population and F:B structure increases. These reductions are potentially due to increases in bacterial and fungal growth efficiencies as observed by del Giorgio and Taylor (del Giorgio & Cole, 1998; Taylor & Townsend, 2010) and are similar to the observations by Fontaine et al. (Fontaine et al., 2007b). The reduction in relative respiration values from ~44% to ~11% of C_{IN}(**Figure 6**) characterizes the potential that higher fertility soils, as defined by SMC with fungal-dominated structures, have for improving carbon-use-efficiency to better retain C compounds in soils as also observed by researchers in Six et al. (Six et al., 2006).

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Field studies demonstrated C_R rates ranging from 1.2 g C m^{-2} $year^{-1}$ to 2.25 g C m^{-2} $year^{-1}$ as C_{IN} ranged from ~0.3% C to 7.2% C, representing at most a doubling of respiration rates in soils demonstrating ~7 times increase in C_{IN} **(Figure 7).** These values (approximately 3.4 times reduction) are in line with Fernandez-Martinez et al. model of nutrient-rich and nutrient-poor forests (Fernandez-Martinez et al., 2014).

Transferability to Field Plots:

Field application of BEAM demonstrates the efficacy of developing SMC population and increasing F:B in agricultural fields through an observed increase in production of Mean Net Primary Productivity (MNPP) as dry aboveground biomass in g C m^{-2} $year^{-1}$. **Figure 8** compares annual MNPP estimates for "Estuaries" ~2,500 g and Tropical Rain Forests" ~2,300 g (two of the earth's most productive ecosystems) to "Cultivated Land" 650 g year (Whittaker, 1975), and the production capacity of two BEAM improved test plots: BEAM-4.5, a 4.5 year application of BEAM and a "BEAM 7.9% C" test plot. The BEAM-4.5 treatment produced annual MNPP ~1,980 g, 1.5 times the MNPP of traditional "Cultivated land", and the "BEAM- 7.9%C" produced up to 8,450 g annual MNPP, 13 times the estimates for "Cultivated Land" and 3.4 time the MNPP of the two most productive ecosystems on earth. These results imply the application of BEAM on agricultural soils may offer an effective and robust mechanism to improve the productivity of agricultural soils, increasing the MNPP of agricultural systems while also reducing the loss of soil carbon through reduction in soil respiration, both being essential for implementing carbon capture and storage in agricultural soils.

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Soil macro and micro-nutrients, along with SMC population and F:B increased during the 19 month field study of initial implementation of BEAM **(Table 3).** No inorganic or synthetic fertilizers were added in the BEAM treatment and no biomass was added or removed from these fields. The increase in macro-, meso- , micro-nutrients and SOM is most likely due to the influence of the SMC population and F:B structural increases. Most soils have the elemental nutrients required by plants but they are not "directly" plant available. Restoration of SMC and soil C (the energy supplies SMC rely on) may enable both the extraction of plant and SMC needed nutrients from the soil parent material and transport these elements to SMC and the plants they associate with.

Conclusions:

BEAM offers an innovative technological approach capable of re-establishing the biogeography of agricultural soils to better emulate the SMC population and F:B structure of healthy natural soil ecosystems. The literature cited in this manuscript relays the research efforts of many scientists and their observations towards understanding how SMC operate in these soil ecosystems. Their efforts have clearly defined the type of soil C that has demonstrated the longest MRT (C structures with microbial signatures) (Chabbi & Rumpel, 2009; Kleber et al., 2011; Miltner et al., 2011; Dungait et al., 2012), the source of this C (SMC utilizing exudates within plant/SMC mutualisms), the structure of the SMC most likely to achieve optimal C production and storage (higher F:B , fungal biomass and microbial signature C) (Wright & Nichols, 2002; Sollins et al., 2009), and the optimal conditions under which SMC achieve the highest carbon use efficiency (higher F:B soils) (Six et al., 2006).

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612 Advanced BEAM soils in this research appear to mimic natural ecosystems through: **1)**
613 increased C capture rates with 3.4 times more biomass production **(Figure 8)** without the use of
614 fertilizers, **2)** increased and improved C storage through enhancement of the plant/SMC
615 mutualisms for increased SMC population and SMC biomass **3)** reduced C_R through improved
616 bacterial and fungal carbon-use-efficiencies along with a shift from plant-signature soil C to
617 microbial-signature soil C within a nutrient rich plant/SMC system. All of these attributes
618 position BEAM agro-ecosystems as the most logical and cost effective path for effective capture
619 and storage of atmospheric carbon (CO_2). The findings in both the greenhouse and field
620 portions of this research characterize the immense potential for capturing and storing large
621 quantities of atmospheric CO_2 in agricultural soils through improvements in soil C and soil F:B.

622 BEAM approaches in low fertility agricultural soils (BEAM-4.5) (C<1.4%C; F:B < 1.0),
623 promoted capture of ~10.27 mt C ha^{-1} yr^{-1}, amounting to approximately 37.7 mt of atmospheric
624 CO_2 ha^{-1} yr^{-1} on soils initiating BEAM. This amount of soil C increase is from 20 to 50 times
625 higher than rates currently observed by other researchers (West, 2002; Lal, 2004; Niggli et al.,
626 2009). If the higher rates of C capture in advanced BEAM soils are considered (4.3 time increase
627 in MNPP comparing BEAM-7.9%C to BEAM-4.5) then 44 mt C ha^{-1} yr^{-1} (162 mt CO_2 ha^{-1} $yr^{-}1$) are
628 realized. This amount, if realized, would capture the entirety of anthropogenic C emissions (9.9
629 x 10^9 mt C $year^{-1}$) on a very small percentage of arable land.

630 The results from the greenhouse experiment in this research gives us a roadmap for the
631 transformations we can expect as we adopt BEAM into agricultural soils. It allows us to better
632 predict plant performance in soils with low C resources, inadequate SMC population and F:B

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633 structures and gives us a realistic estimate of the true capacity of what plants and SMC can
634 accomplish when allowed to function under optimal conditions. Preliminary costs for adopting
635 BEAM practices on agricultural soil is estimated to be ~$17-$18 ton^{-1} CO_2, (to cover farmer's
636 seed, cultivation, water and labor costs) or approximately 1/10 the estimated current cost of
637 Carbon Capture and Storage ($49-$110 ton^{-1} CO_2) (Middleton & Brandt, 2013). Offset costs for
638 CO_2 capture using BEAM would amount to a 6% surcharge on all consumer energy products.
639 ($0.16-$0.18 gal^{-1} of gasoline or diesel, $2.49 for the average airplane flight [~2300 km @ 75g
640 CO_2 km^{-1}, 79% occupancy] less than the cost of a drink on that flight, or ~$0.01 kWhr^{-1} added to
641 the cost of electricity)

642 Besides for capturing large amounts of atmospheric CO_2 and restoring soil carbon
643 reserves, along with the SMC that thrive on them, application of BEAM will: **1)** reduce fertilizer
644 usage and its associated downstream pollution of our aquifers, lakes, rivers, estuaries, oceans
645 and coral reefs, and **2)** reduce the quantity of water required to grow crops- (currently 80% of
646 the world's water resources are used for agriculture) by increasing soil water infiltration, soil
647 water retention, plant-water and plant-nutrient-use efficiencies for more efficient plant growth
648 and **3)** decrease on-farm energy use through adoption of lower-impact reduced-energy
649 agricultural management methodologies.

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Figures and Tables:

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Figure 1– The stacked columns represent the carbon partitioning (g) of New Soil C, Root C, Shoot C, Fruit C, Respiration C for each treatment Fungal:Bacterial Ratio (F:B) as designated by key. The line with markers represents the "% of Total New System C Diverted to Soil".

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Table 2: Schedule of planting, harvesting and sampling schedule for BEAM-4.5 (1.52%C) indicating aboveground biomass (mt C/Acre) and associated soil C (%C).

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852 **Figure 5**: Comparison of the Fungal:Bacterial (F:B) ratio after 19 months adoption of BEAM on
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854 managed agriculture treatment.

855 **Figure 6**– Greenhouse experiment Soil C respiration (%) compared to Initial Soil C (CIN) content
856 (g).

Figure 7– Soil C respiration (g C m^{-2} day^{-1}) for a one year’s sampling (9/2010-8/2011 with 32 sampling events) of 4 field soil treatments and a desert soil plot (Desert, Control, Conv, BEAM-4.5 and BEAM-7.6%C). The vertical lines represent the maximum and minimum respiration measurements (g C m^{-2} day^{-1}) recorded over the duration of the one year’s sampling. The outlined rectangles represent respiration measurements within the 20th quintile to the 80th quintile, or the recorded range of 60% of the respiration measurements. The grey shaded rectangles represent the soil C% of each of the field treatments (C%). These were included to portray the significance in the difference of soil C percentages relative to the lesser differences in the respiration measurements recorded in g C m^{-2} day^{-1}.

Figure 8: Mean Net Primary Production (MNPP) (g dry aboveground biomass m-2 yr-2) of three different ecosystems, Kelp Beds and Reefs, Tropical Rain Forests and Cultivated Land (Whittaker, 1975), as compared to two BEAM plots, BEAM-4.5 and BEAM-7.9%C treatment.

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Supplementary Information

S1-Figure 1: Soil microbial community analyses of the compost used to mix soil treatments 0-5 analyzed by Soil Foodweb Oregon LLC 635 SW Western Blvd, Corvallis OR 97333 to enumerate fungal, bacterial, protozoan and nematode populations.

S1-Figure 2: Soil microbial community analyses of the alluvial sand used to mix soil treatments 0-5 analyzed by Soil Foodweb Oregon LLC 635 SW Western Blvd, Corvallis OR 97333 to enumerate fungal, bacterial, protozoan and nematode populations

S1-Figure 3: Results of static alkali reactor sensitivity analyses (with 1, 2 and 3 day reactor operating times) conducted to confirm CO2 absorption characteristics, variance and reproducibility with different reaction times.

S1-Figure 4: Results from a GLM regression analysis, comparing initial calculated treatment soil mix (C%) with mass Loss on Ignition analyses to confirm experimental setup, produced a linear trend line with an r2=0.98 (P= 0.0002).

S1-Figure 5: Trend line analyses for Carbon (g) partitions : Shoot C, Fruit C, Root C, New Soil C , Total Plant C, Total New System C and Respiration C as compared to Fungal:Bacterial Ratio (F:B)

S1-Figure 6: Trend line analyses for Nitrogen (g) partitions: Plant Canopy N, Chile N, Root N, New Soil N, Total Plant N and Total System New N.

S2-Tables 1- Soil and compost mix data, soil carbon and nitrogen metrics, microbial metrics and ending soil, root, shoot and fruit C and N mass and %.

S2-Tables 2- Values and statistics for Ending Soil Dry Mass.

S2-Tables 3- Values and statistics for Ending Soil Carbon %.

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S2-Tables 4- Values and statistics for Ending Soil Nitrogen %.

S2-Tables 5- Values and statistics for Shoot Mass.

S2-Tables 6- Values and statistics for Fruit Mass.

S2-Tables 7- Values and statistics for Root Mass.

S2-Tables 8- Values and statistics for Plant Component C% and N%.

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Figure 1(on next page)

Tables and Figures

Table 1

Treatment	0	1	2	3	4	5
Beginning Soil Metrics						
Sand (g dry)	1465.86	1221.55	977.24	732.93	488.62	244.31
Compost (g dry)	0	96.73	193.47	290.2	386.94	483.67
Total Dry Mass (g)	1465.86	1318.29	1170.71	1023.14	875.56	727.98
Initial Soil C%	0.14%	0.71%	1.42%	2.34%	3.57%	5.30%
Initial Soil N%	0.01%	0.05%	0.11%	0.18%	0.27%	0.40%
Initial Soil C (g)	2.05	9.36	16.67	23.98	31.29	38.6
Initial Soil N (g)	0.15	0.7	1.26	1.81	2.37	2.93
Beginning Microbial Metrics						
Bacteria (g reactor^{-1})	0.313	0.321	0.329	0.337	0.344	0.352
Fungi (g reactor^{-1})	0.011	0.269	0.527	0.784	1.041	1.299
Total F:B Ratio	0.04	0.84	1.6	2.33	3.02	3.68

Table 1: Initial soil mass, soil C, soil N, and Soil Microbial Community metrics for the greenhouse portion of this research.

Figure 1

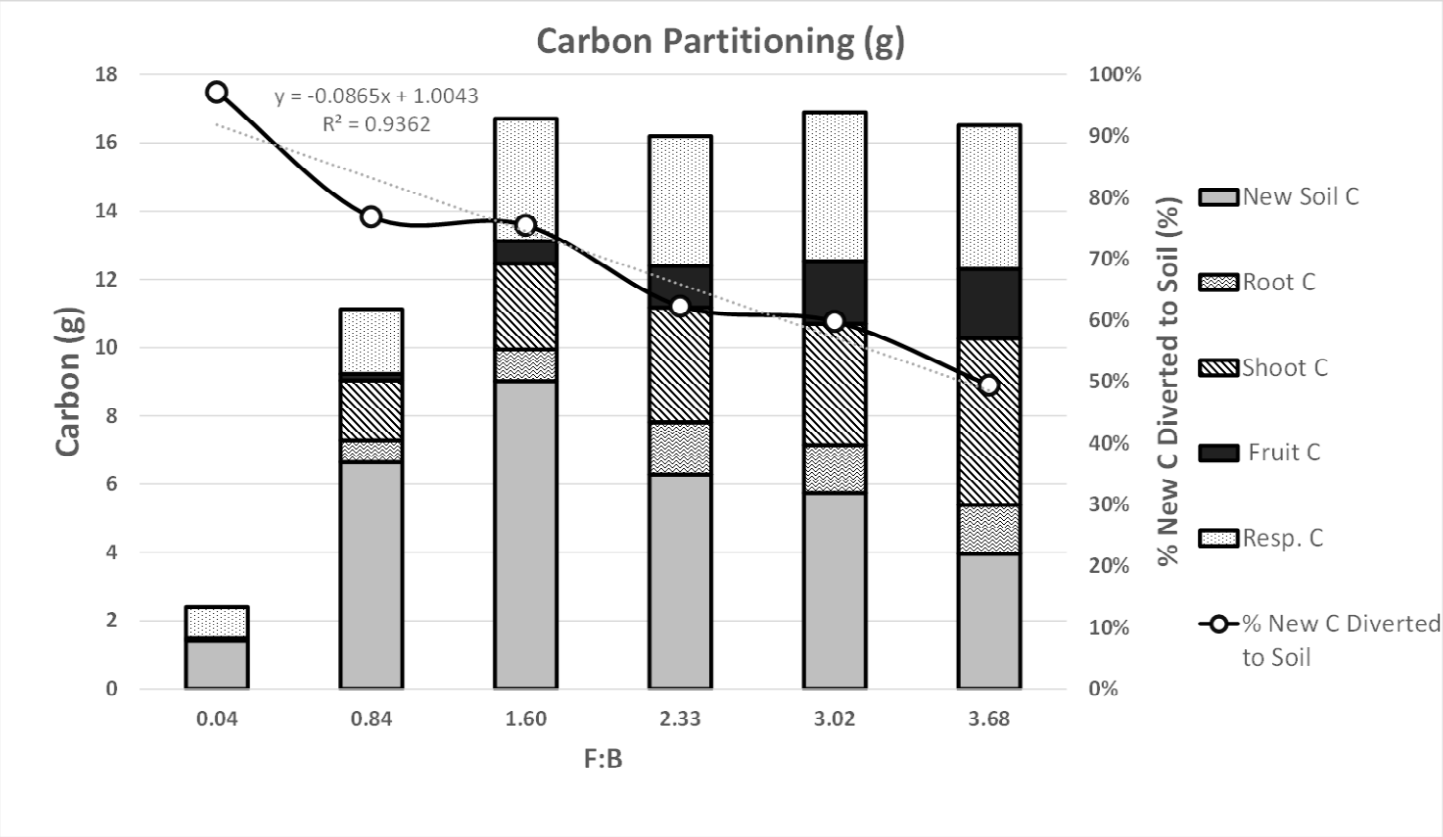

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Figure 2

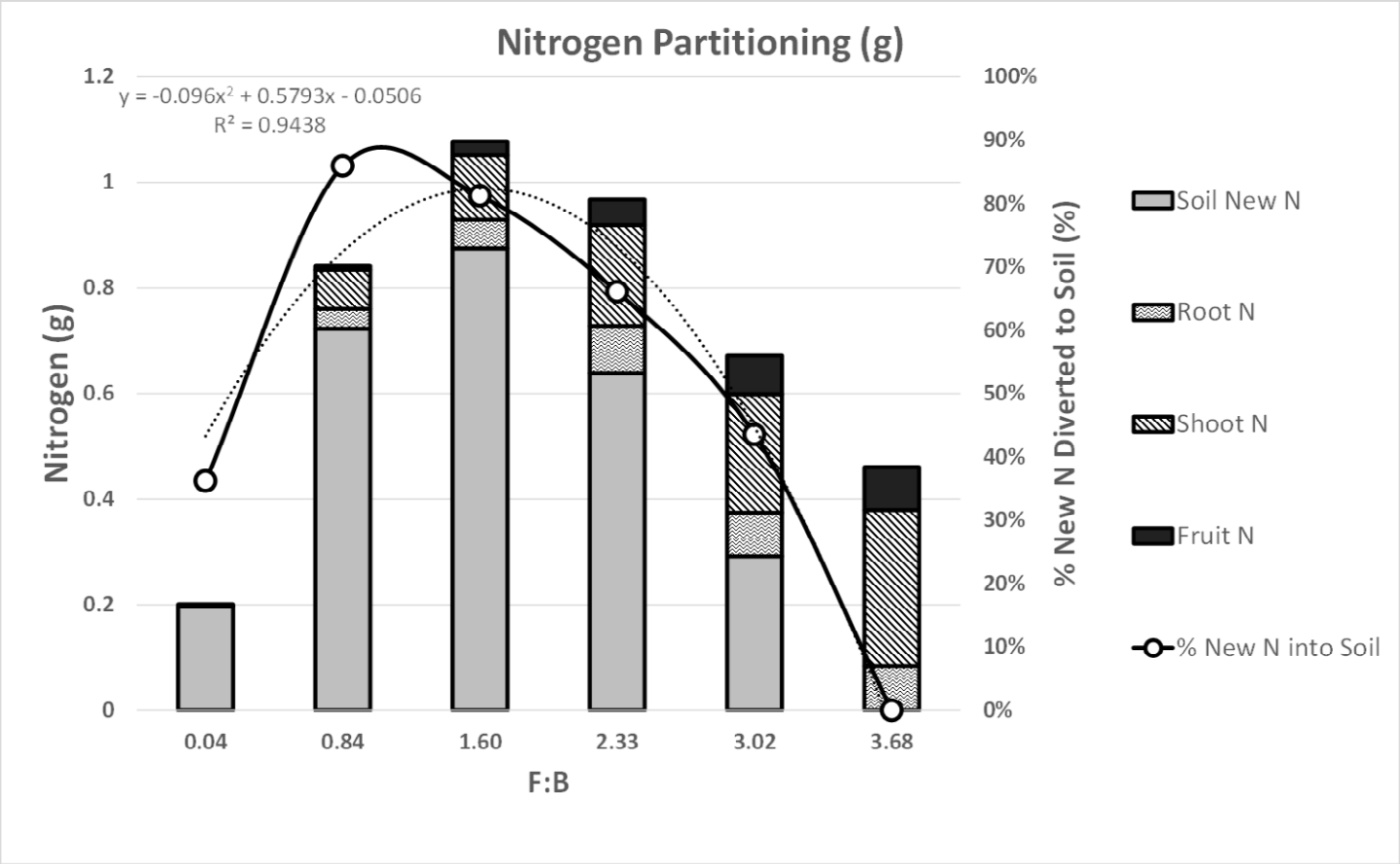

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Table 2

Month	Date (m/yr)	Crop/Action	Aboveground Biomass (mt/ha)	Soil Carbon (%C)
0	9/2009	Cover Planted		0.43 %C
9	**5/2010**	**Cover Harvest**	**2.71**	
12	7/2010	Cover Harvest	3.10	
15	**9/2010**	**Cover Harvest/S.T.**	**5.57**	**0.73 %C**
15	11/2010	Soil Test		0.71 %C
20	**12/2010**	**Cover Harvest**	**4.94**	
22	6/2011	Soil Sample		0.87 %C
25	**9/2011**	**Cover Harvest**	**1.89**	
26	10/2011	Soil Sample	4.26	
32	**4/2012**	**Cover Harvest**	**6.44**	
37	9/2012	Cover Harvest	4.02	
44	**4/2013**	**Cover Harvest**	**3.34**	
56	4/2014	Chile	4.02	1.52 %C
Total			**40.29**	**Delta C% = 1.09%**

Table 2: Schedule of planting, harvesting and sampling schedule for BEAM-4.5 (1.52%C) indicating above-ground biomass (mt C/Acre) and associated soil C (%C).

Table 3

Months	0	6	8	15	19	Percent Increase	R^2	Regression
Calcium (meq/L)	4.09	2.82	3.00	6.07	7.19	75.79%	$R^2 = 0.6367$	Linear
Copper (mg/kg)	**1.17**	**1.1**	**1.04**	**1.74**	**1.64**	**40.17%**	**$R^2 = 0.6591$**	**Linear**
Iron (mg/kg)	4.89	4.12	2.66	27.01	59.19	1110%	$R^2 = 0.9892$	2nd Order
Potassium (mg/kg)	**30**	**33**	**32.00**	**42**	**41**	**37%**	**$R^2 = 0.8712$**	**Linear**
Kjeldahl N (mg/kg)	633	719	739.00	752	1041	64%	$R^2 = 0.8244$	2nd Order
Magnesium (mg/kg)	**1.09**	**0.075**	**0.81**	**1.67**	**1.99**	**83%**	**$R^2 = 0.7954$**	**2nd Order**
Manganese (mg/kg)	3.25	1.86	1.65	14.31	40.14	1135%	$R^2 = 0.969$	2nd Order
NO_3-N (mg/kg)	**1.5**	**1.55**	**2.00**	**2.35**	**3.1**	**107%**	**$R^2 = 0.9847$**	**Linear**
Phosphorus (mg/kg)	6.9	12.2	10.00	15.3	11.3	64%	$R^2 = 0.4624$	Linear
Zinc (mg/kg)	**0.5**	**0.63**	**0.48**	**0.93**	**0.81**	**62%**	**$R^2 = 0.6652$**	**Linear**
SOM (%)	0.75	1.25	1.22	1.49	1.41	88%	$R^2 = 0.7854$	Linear

Table 3: Soil macro and micro-nutrient analysis observing soil element changes in the initial (19) nineteen month period from adoption of a BEAM approach on BEAM-4.5. Column "% Increase" indicates the amount of positive change in soil elemental change over the 19 month period. Column "R^2" and "Regression" indicates the statistical r-square and regression trend line characteristics.

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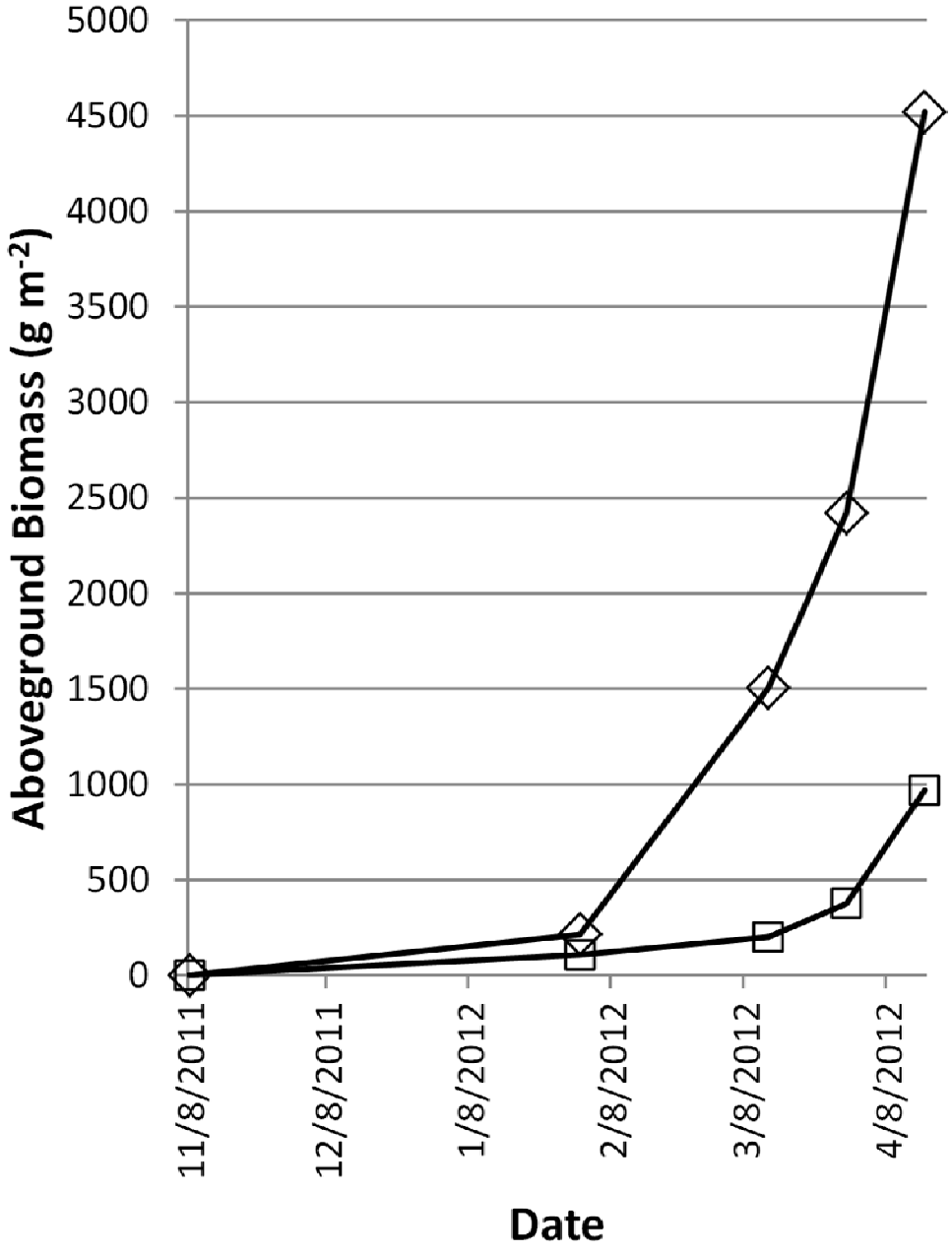

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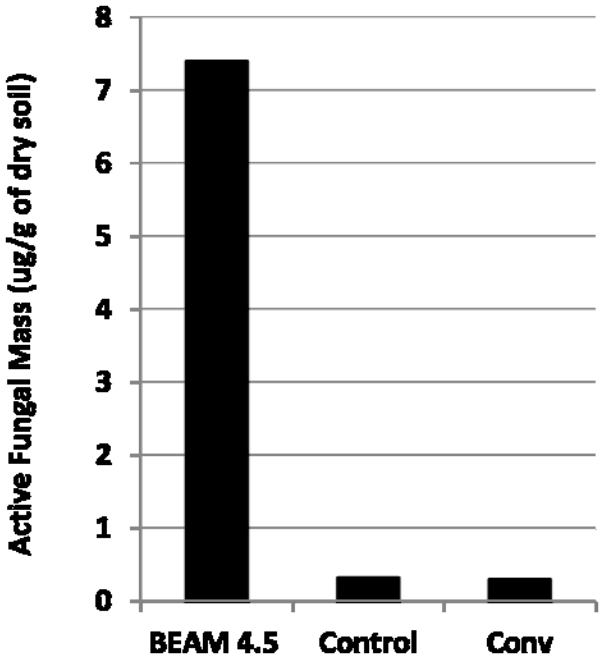
Figure 4



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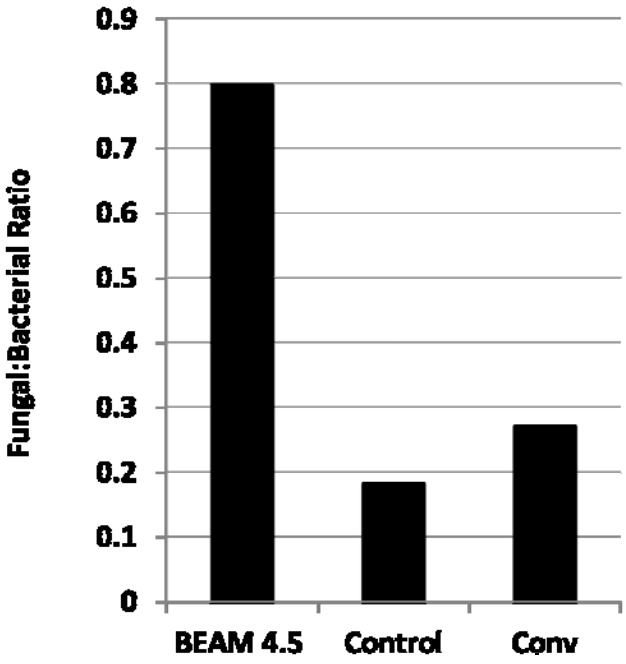

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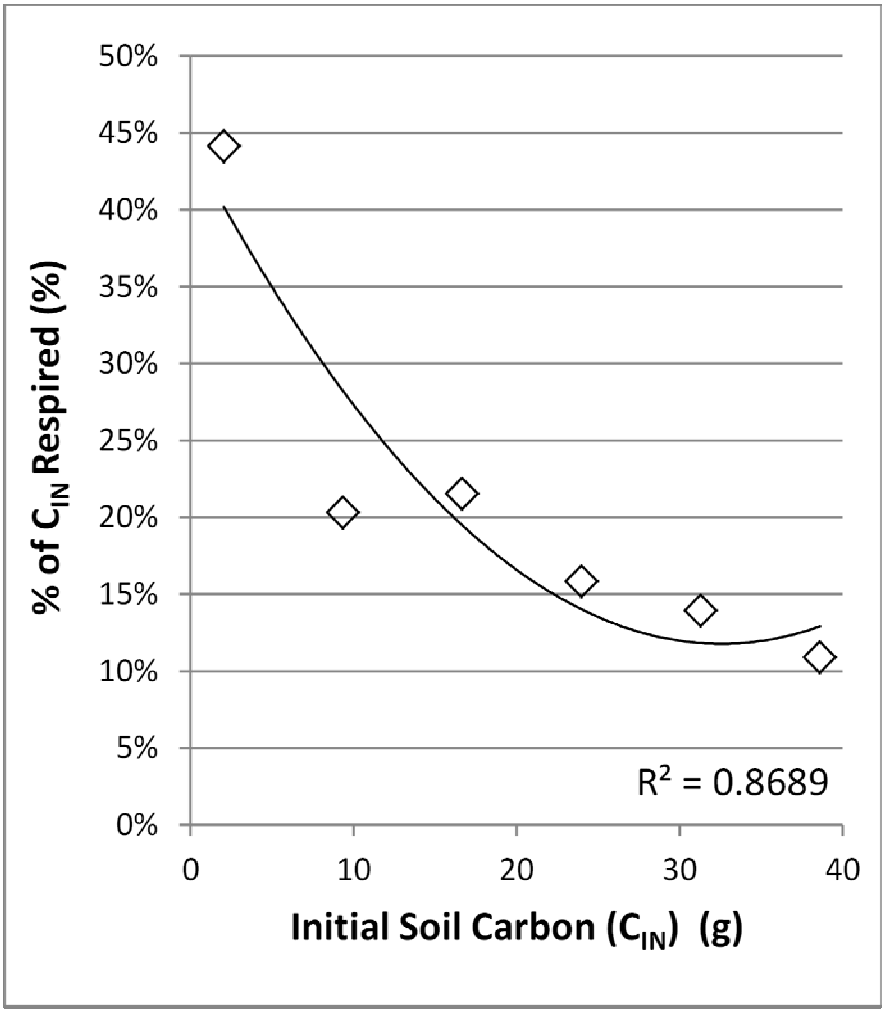

Figure 6– Greenhouse experiment Soil C respiration (%) compared to Initial Soil C (C_{IN}) content (g).

Figure 7

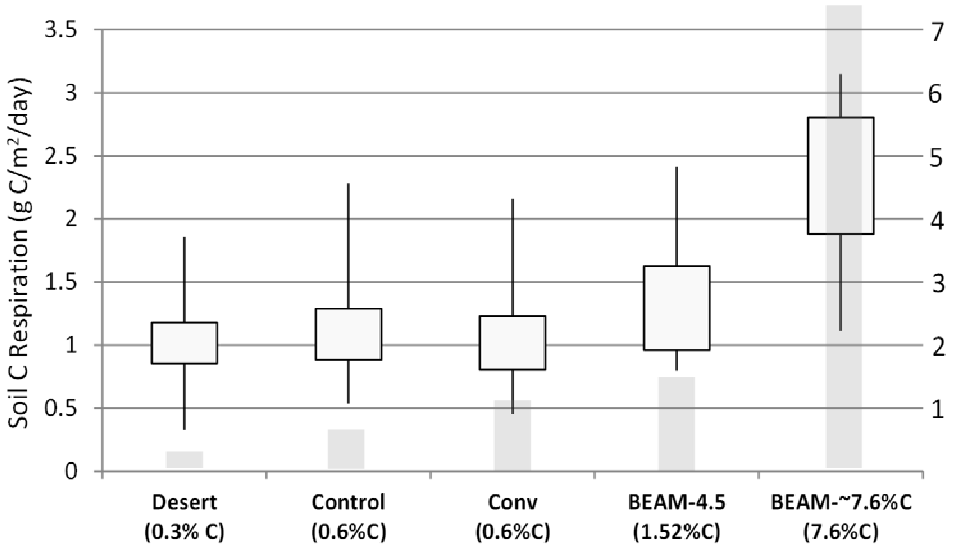

Figure 7: Soil C respiration (g C m^{-2} day^{-1}) for a one year's sampling (9/2010-8/2011 with 32 sampling events) of 4 field soil treatments and a desert soil plot (Desert, Control, Conv, BEAM-4.5 and BEAM-7.6%C). The vertical lines represent the maximum and minimum respiration measurements (g C m^{-2} day^{-1}) recorded over the duration of the one year's sampling. The outlined rectangles represent respiration measurements within the 20th quintile to the 80th quintile, or the recorded range of 60% of the respiration measurements. The grey shaded rectangles represent the soil C% of each of the field treatments (C%). These were included to portray the significance in the difference of soil C percentages relative to the lesser differences in the respiration measurements recorded in g C m^{-2} day^{-1}.

Figure 8

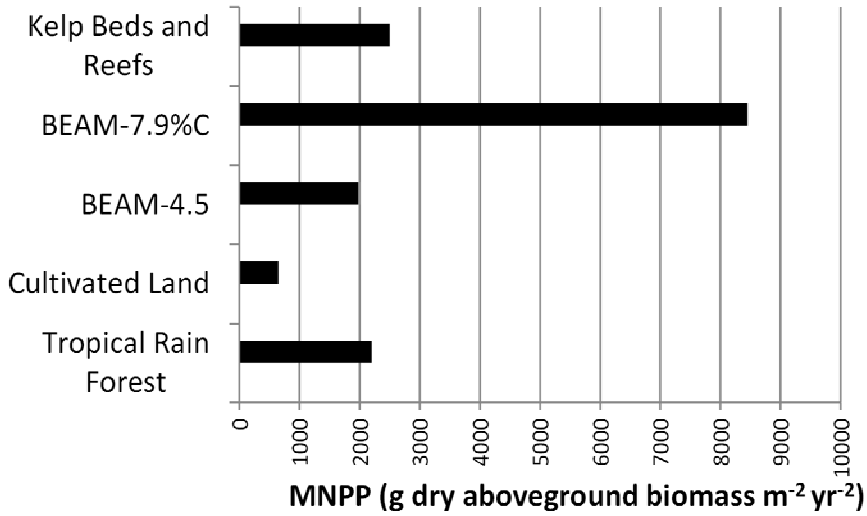

Figure 8: Mean Net Primary Production (MNPP) (g dry aboveground biomass m^{-2} yr^{-2}) of three different ecosystems, Kelp Beds and Reefs, Tropical Rain Forests and Cultivated Land (Whittaker, 1975), as compared to two BEAM plots, BEAM-4.5 and BEAM-7.9%C treatment.