The influence of soil microbial community structure on carbon and nitrogen partitioning in plant/soil ecosystems

David C. Johnson Corresp. 1

1 Institute for Sustainable Agricultural Research, WERC, College of Engineering, New Mexico State University, Las Cruces, NM, USA

Corresponding Author: David C. Johnson
Email address: davidcjohnson@nmsu.edu

A greenhouse study was conducted to evaluate the influence of increasing soil fungal-to-bacterial ratios (F:B) on the allocation of plant-photosynthate carbon into the carbon (C) and nitrogen (N) partitions (g) of plant components (root, shoot and fruit), New-Soil C and N, and Soil-Respiration C (CO_2). Six (6) experimental treatment soils were formulated to provide linearly increasing: initial-soil C% (0.14% – 5.3%); initial-soil N% (0.01% - 0.40%); and soil microbial community (SMC) populations progressing from bacterial dominant (F:B=0.04) to fungal dominant (F:B=3.68) while still maintaining significant SMC population homogeneity. In an 86-day greenhouse experiment, growing chile plants (*Capsicum annuum*) in treatment soils with increasing F:B (0.4-3.68), the following was observed: **a)** a continuous linear increase (3% up to 56%) in the partitioning of total plant-photosynthate C into plant biomass (root, shoot and fruit) when regressed to initial F:B (m=0.13; r^2=0.96); **b)** approximately 93% of the flow of plant-photosynthate C was partitioned into New-Soil C in Treatment 0 (F:B = 0.04), to a minimum of 47% in Treatment 5 (F:B = 3.68) demonstrating a negative linear correlation to treatment Initial-Soil C mass (m= -0.12; r^2 = 0.97); **c)** conditional and coordinated flow of system C resources into nitrogen (N) fixation (est. C cost for N fixation at 6:1), with 1.21 g C partitioned to N fixation in Treatment 0 (F:B=0.04), peaking at 6.92 g C in Treatment 2 (F:B=1.6), and final C partitioning to N fixation of 2.91 g C in Treatment 5 (F:B=3.68), following a 3rd order polynomial trendline (r^2=0.99) when correlated with initial treatment soil C mass; **d)** decreases in soil respiration, from 44% of Initial-Soil C substrate respired in bacterial-dominant low-C (0.14%) soils (F:B = 0.04) to 11% in fungal dominant (F:B = 3.68), high-C percent (5.30% C) soils (y = -0.108ln(x)+ 0.4987; r^2= 0.95). Increasing the F:B in the soils of agroecosystems may provide more efficient accumulation and partitioning of photosynthate C into plant and soil biomass, improved N fixation and beneficial increases in total carbon use efficiencies. Collectively, these benefits could provide a practical and cost-effective path towards: improving crop production, reducing N-fertilizer inputs, promoting a more sustainable agricultural system, while providing a cost-effective

approach for capturing and storing atmospheric carbon (CO_2) in soils of agroecosystems.

Title: **The Influence of Soil Microbial Community Structure on Carbon and Nitrogen Partitioning in Plant/Soil Ecosystems**

Author: David C. Johnson[1*]

Affiliations: [1*] Institute for Sustainable Agricultural Research; WERC; College of Engineering, New Mexico State University, Las Cruces, NM 88003, USA

*Correspondence to: davidcjohnson@nmsu.edu Phone: 575-646-4163

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Abstract: A greenhouse study was conducted to evaluate the influence of increasing soil fungal-to-bacterial ratios (F:B) on the allocation of plant-photosynthate carbon into the carbon (C) and nitrogen (N) partitions (g) of plant components (root, shoot and fruit), New-Soil C and N, and Soil-Respiration C (CO_2). Six (6) experimental treatment soils were formulated to provide linearly increasing: initial-soil C% (0.14% – 5.3%); initial-soil N% (0.01% - 0.40%); and soil microbial community (SMC) populations progressing from bacterial dominant (F:B=0.04) to fungal dominant (F:B=3.68) while still maintaining significant SMC population homogeneity. In an 86-day greenhouse experiment, growing chile plants (*Capsicum annuum*) in treatment soils with increasing F:B (0.4-3.68), the following was observed: **a)** a continuous linear increase (3% up to 56%) in the partitioning of total plant-photosynthate C into plant biomass (root, shoot and fruit) when regressed to initial F:B (m=0.13; r^2=0.96); **b)** approximately 93% of the flow of plant-photosynthate C was partitioned into New-Soil C in Treatment 0 (F:B = 0.04), to a minimum of 47% in Treatment 5 (F:B = 3.68) demonstrating a negative linear correlation to treatment Initial-Soil C mass (m= -0.12; r^2 = 0.97); **c)** conditional and coordinated flow of system C resources into nitrogen (N) fixation (est. C cost for N fixation at 6:1), with 1.21 g C partitioned to N fixation in Treatment 0 (F:B=0.04), peaking at 6.92 g C in Treatment 2 (F:B=1.6), and final C partitioning to N fixation of 2.91 g C in Treatment 5 (F:B=3.68), following a 3rd order polynomial trendline (r^2=0.99) when correlated with initial treatment soil C mass; **d)** decreases in soil respiration, from 44% of Initial-Soil C substrate respired in bacterial-dominant low-C (0.14%) soils (F:B = 0.04) to 11% in fungal dominant (F:B = 3.68), high-C percent (5.30% C) soils (y = -0.108ln(x)+ 0.4987; r^2= 0.95).

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1.0 Introduction

Soil organic matter (SOM), and the carbon and nutrients it contains, are key components for supporting fundamental bio-geochemical processes for: carbon assimilation, plant growth, soil respiration, and carbon-climate feedbacks (Kallenbach, Frey, & Grandy, 2016). Soil microbial community (SMC) population, structure, and biological functionality facilitate these bio-geochemical processes and contribute substantially to: nutrient cycling, nutrient capture, soil fertility development, and SOM formation and turnover. (Schloter et al., 2003; Van der Heijden et al., 2008; Murray et al., 2009; Garcia-Orenes et al., 2016).

A shift towards fungal dominance in SMC is believed to enhance C accumulation and reduce SOM turnover rates (Six *et al*., 2006). More efficient microbial biomass production and the accumulation of SOM are now considered to be driven by distinct microbial community structures, where microbial-derived SOM is greatest in soils that contain higher fungal abundances (Kallenbach, Frey, & Grandy, 2016).

Predicting the effects of SMC physiological regulation on soil C processes and their interaction with plants is critical if we are to improve the performance of our agroecosystems, project future global warming potentials, and begin reducing atmospheric CO_2 (Billings and Ballantyne, 2013). Despite this expectation, many studies have concluded there is no direct evidence that soil fungal-to-bacterial ratios (F:B) characterize the turnover of soil organic matter (Rousk & Frey, 2015), soil nutrient content or growth of vegetation (Wong et al., 2015), or that fungi are capable of enhancing soil carbon storage mechanisms (Thiet, Freya & Six,

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2012). Detailed understanding of the SMC relationship to ecosystem function has often proven to be complicated, as have the development of methods to accurately assess them. Much of this difficulty is due to our inability to make direct observations, the technical challenges in measuring *in situ* activities, and the high diversity and/or spatial heterogeneity of these SMC, (Barns et al., 1999; Torsvik et al., 2002; Strickland and Rousk, 2010; Malik et al., 2016).

1.1 Research Focus

To address these issues, a greenhouse experiment was designed to reduce the influence of SMC population heterogeneity to promote a better understanding of the relationship and influence of SMC population and structure on plant growth and carbon partitioning. The focus of the present research was to investigate how increases in soil F:B, while maintaining significant homogeneity of the SMC population, influence the formation and stabilization of C and N in the partitions of: the roots, shoots, and fruit of a plant, newly placed soil C, C partitioned into N fixation, and C respired from the soil. The hypothesis tested in this research is: the F:B of a SMC, influences plant biomass productivity and promotes selective partitioning of C into plant, soil and atmospheric sinks and N fixation.

2.0- Materials and Methods

2.1- Soil Carbon, Nitrogen and Microbial Community Analyses

Initial soil profiles of TC%, TN% and gravimetric analysis for the soils used in the greenhouse trials of this research, were performed using LECO analysis by the Soil, Water and

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Forage Analytical Laboratory at Oklahoma State University. Samples of these same two soils was sent to Earthfort Labs, Soil Foodweb Oregon LLC, 635 SW Western Blvd, Corvallis, OR 97333, for SMC biomass analyses to quantify fungal and bacterial biomass through sample preparation, staining procedures, and biomass quantification using direct observation (microscopy) and other methodologies (Ingham, 1995; Ekelund, 1998; Stamatiadis et al., 1990). Laboratory results for the analyses of the SMC components of the two soil (compost and alluvium soils) used in this study are in the supplementary material **(S1-Figure 1, S1-Figure 2**).

2.2- Greenhouse Experiments

Greenhouse experiments were designed to quantify the growth characteristics of chile plants (*Capsicum annuum*, variety Big Jim "Heritage") in six soil treatments mixed for linearly increasing: Initial–Soil C (g) and N (g) content; SMC biomass (µg g^{-1} dry soil), and F:B **(Table 1).** Treatment soils were formulated from the mixing of two soil components. The first was a compost product with a fungal-dominant SMC structure (F:B=4.62), C%=7.91%; N% = 0.60%; obtained from a Johnson-Su composting bioreactor (Johnson & Su-Johnson, 2010) **(S1-Figure 1**). The design of the Johnson-Su bioreactor promotes development of a compost product with a high F:B ratio, permitting the formulation of a wide range of initial treatment soil F:B implemented in this research. The second soil was a bacterial-dominant soil (F:B 0.04); C%= 0.14%; N%= 0.01%; an arroyo alluvium (Bluepoint-Caliza-Yturbide complex, predominantly sand, loam, and clay (at compositions of 76%, 20%, and 4%, respectively) obtained from a local desert arroyo **(S1-Figure 2)**. Soils in the six greenhouse experimental treatments (0, 1, 2, 3, 4, 5) were formulated, based on both dry and wet-mass-ratio mixtures of these two soil

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components, to demonstrate linearly increasing percentages of initial soil C% (0.14% and 5.3%), N% (0.01% and 0.4%), and associated SMC metrics, including a shift in F:B from a bacterial-dominant soil to a fungal-dominant soil (0.04 and 3.68) **(Table 1)**. The investigated ranges of F:B in this experiment encompass current soil conditions in conventional agriculture, low C (0.14%C) and bacterial-dominant (F:B=0.04); to a fertile soil condition envisioned for healthy agroecosystems, demonstrating increased soil C content (5.30% C) and a shift in the structure of the SMC towards fungal-dominance (F:B=3.68).

Chile seeds (*Capsicum annuum*, Big Jim "Heritage" variety) were planted in each of the six treatments (0, 1, 2, 3, 4 and 5) (n = 5 in each treatment), and a single blank for each of the six treatments with no plant. Planted containers were allowed to grow from seedling to harvest in an 86-day growth period. Four seeds were planted in each 1.325 liter plastic plant container and thinned to 2 healthy plants per container approximately 10 days after germination. Plant containers were watered daily with approximately 50-75 mL of distilled water taking care to not have any excess flow of water, and/or sediment through treatment pots. Photosynthetically active radiation was supplied by two, 2' x 4' SlimStar, 6-bulb, high-output T-5 grow lamps with 30,000 lumens/fixture (6,400 K spectrum Grow bulbs), operating for 12 hours per day for the 86-day growing period.

After the growing period, root biomass was removed from the soils in all treatment plant containers, and then the mass and water content of each treatment soil was determined. Subsamples of the soils from each of the treatment plant containers (0-5) were individually pooled and thoroughly mixed, and then shipped to the Oklahoma State University Soils and

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Water Testing Laboratory to be analyzed in triplicate for total C% and total N% as well as soil moisture content (%). Plant tissues (roots, shoot, and fruit) were harvested separately, oven-dried for 3 days at 45 ºC in pre-weighed oven-dried paper bags, and then re-weighed to the closest 0.0001 g on a Mettler AE200 balance. Composite samples of plant tissues (root, shoot and fruit) were shipped to the Oklahoma State University Soils and Water Testing Laboratory to be analyzed in triplicate for TC% and TN%. Raw data for initial treatment soil fabrication matrix C, N percentage and mass, and SMC community mass is represented in **Table 1.** Raw data and ANOVA results for final treatment soil mass, plant component mass, plant and soil C% and N%, and Soil-Respiration C mass are in **S1-Table 1a-c**, **S1-Table 2a-c**, and **S1-Table 3a-c**.

2.3- Loss on Ignition Analysis

To verify accuracy for the mixing protocols of the two soil components, relative to the initial experimental treatment design matrix, an initial soil C% analysis was conducted on each of the treatment soils (0-5)using loss-on-ignition (LOI) soil analyses to determine treatment soil organic carbon percentages (SOC%). Triplicate soil samples (~6-10 g) representing each treatment were pre-weighed, dried overnight at 105 ºC in a muffle furnace, weighed again for dry mass, and then subjected to a follow-up LOI analysis for 2 hours at 375 ºC to avoid potential decomposition of inorganic carbonates present in area soils (Schumaker, 2002). A final weighing and calculation of soil mass LOI (g) was performed and these values were regressed against the Initial-Soil C% content calculated in the original soil fabrication design matrix (**Table 1)**.

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2.4- Soil Respiration

Reliable methodologies for ensuring the accurate measurement of soil CO_2 efflux are still under debate and development (Pumpanen et al., 2004; Kuzykov, 2010); therefore, due to methodological sensitivity, and repeatability; and the treatment plant container size (1.325 L), a static alkali trap methodology was chosen to measure Soil-Respiration C.

Alkali traps can yield overestimates of low CO_2 fluxes and underestimates of high CO_2 fluxes, but they can be reliably calibrated for intermediate ranges of CO_2 flux (Davidson et al., 2002). Accurate soil respiration measurements can be affected when the insertion of sampling collars severs root structures, when only daytime measurements are taken (Heinemeyer et al., 2011), and when the surface area of the alkali reaction vessel is less than 6% of the soil surface area sampled (Raich & Nedelhoffer, 1989). Parameters for the proper use of static alkali reactors in this research followed all of these accepted methodological guidelines to ensure accurate soil respiration measurements. While not an absolute quantitative assessment, the static alkali reactor systems were able to render a reliable, internally comparable analysis of soil CO_2 emissions, as well as soil respiration values (g C m^{-2} day^{-1}) within the historically observed ranges when compared to different ecosystems and types of vegetation (Raich & Schlesinger, 1992).

A preliminary soil C respiration experiment was conducted to verify the sensitivity of static alkali reactors in 1, 2 and 3 day test durations and across the range of soil C% and anticipated experiment CO_2 production levels. A single-factor ANOVA analysis defining the

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sensitivity parameters, yielded low variances (<0.05) and accurate repeatability for tests of 1, 2 and 3 days in duration, throughout the range of experimental treatment Initial-Soil C substrate masses (**S1-Figure 3**).

Soil-Respiration C (g), for each treatment in the greenhouse trials, was measured with static alkali reactors comprised of a 50 mL plastic centrifuge tube, containing 15 mL of standardized 1 *M* KOH, with a cross-sectional area of about 25% of soil surface sampled. Reactors were covered with a 1 liter glass cover (canning jar) pushed approximately 3 cm deep into the soil and allowed to remain undisturbed for a 24-hour period to quantify soil respiration over a diurnal period. The 50 mL tubes were then removed from the reactors, capped, and taken to a laboratory for titration analyses and C respiration calculation according to USDA/ARS in-situ chamber techniques for the measurement of soil respiration (Rochette & Hutchinson, 2005).

Soil-Respiration C (g) measurements were conducted on each of the experimental treatments (0-5 and the blank) at selected intervals (4 separate samplings on days 38, 46, 58, and 86 after seeding) using a non-repeating, randomly-chosen plant container in each treatment over the 86 day growth period (**S1-Table 3c**). Observed treatment respiration rates were adjusted for static alkali reactor soil-surface area and treatment container soil-surface area to calculate CO_2 respiration rates (g C m^{-2} d^{-1}) for each measurement period (**S1-Figure 5f**). Soil-Respiration C (g), for each soil treatment, was estimated using a cumulative assessment of treatment respiration rates for the number of days occurring previous to each of the four sampling time periods, and the planting pot surface area, summing the Soil-Respiration C (g)

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values of each of the sampling intervals to yield total C (g) respired over the 86 day growth period, planting to harvest, for each treatment.

2.5- Statistical Analyses

Single-factor analysis of variance (ANOVA) was conducted to analyze treatment ending: root mass (g), shoot mass (g), fruit mass (g), ending soil dry mass (g), ending soil C% and N%, plant component (root, shoot and fruit) C% and N% percent, and cumulative soil respiration (**S1-Tables 1a-c, 2a-c, and 3a-c** respectively). Statistical averages of this data, from each of the reactors in each of the six treatments, was analyzed with Excel 2010 Analysis ToolPak Add-in using Generalized Linear Model (GLM) and polynomial regression techniques, to quantify and compare the partitioning of C (g) and N (g) mass into plant and New-Soil C partitions, and Soil-Respiration C (g), in the six soil treatments [Treatments 0, 1, 2, 3, and 5 (n=4) and Treatment 4 (n=3)] (**S1-Tables 1a-c, 2a-c, and 3a-c).** An average of one reactor in each treatment was excluded due to inadequate plant germination, insect damage, and/or soil loss events that would lead to inaccurate assessment in experiment mass-balance calculations.

3.0- Results

3.1- Greenhouse Experiments

3.1.1- Treatment Soil-Mixture Matrix Verification

A GLM analysis of the data from LOI analysis (n=3), of the initial soil samples from all 6 treatments (0-5), was regressed against the matrix-derived treatment soil C mass of the two-

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component soil mixture (compost / alluvial sand) to assess the validity of the mixing protocol and to determine the accuracy of beginning-treatment soil C percentages. This GLM analysis produced a linear trend line (r^2 = 0.98; P = 0.0002) **(S1-Figure 4).**

3.1.2- Carbon Partitioning

Carbon mass (g) was evaluated in seven partitions for each treatment: Root C, Shoot C, Fruit C, Initial-Soil C, New-Soil C, Soil-Respiration C, and Total-System-New C, a summation of all new and/or replacement soil carbon (i.e. respiration) in the preceding partitions. Carbon mass for each C partition in each treatment was based on the average values derived from single-factor ANOVA analyses of plant and soil mass data available in **S1-Tables 1a-c, 2a-c, and 3a-c**.

The mass of the individual plant C partitions, Shoot-C, Fruit-C, and Root-C, exhibited positive linear regression trend lines (r^2 *= 0.95, 0.99, and 0.83*, respectively) when regressed with initial soil F:B **(S1-Figures 5a, 5b, and 5c**) (Comparison of C partitions to initial soil F:B and/or Initial-Soil C are synonymous, as initial treatment mixtures were designed to exhibit linear increases in both F:B and Soil C and N mass; this will be discussed further in the Discussion). Collectively, as Total-Plant C, these partitions exhibited a positive linear trend with r^2 *= 0.98* (**S1-Figure 5d**) when regressed with initial soil F:B.

Carbon partitioning into treatment New-Soil C partitions (**S1-Figure 5e**) was 0.06 g C in Treatment 0 (F:B=0.04), increasing up to 7.73 g C in Treatment 2 (F:B = 1.60), after which there was a steady decrease from 6.73 g C in Treatment 3 to 3.97 g C in Treatment 5 (F:B = 3.68).

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Partitioning of C into New-Soil C exhibited a 3rd-order polynomial trend (r^2 = 0.97) **(S1-Figure 5e)** when regressed with initial soil F:B.

Carbon partitioning in treatment Soil-Respiration C partitions (**S1-Figure 5f**) was 0.91 g C in Treatment 0 (F:B=0.04) increasing steadily and leveling off to approximately 4.28 g C in Treatment 4 (F:B=3.02) and Treatment 5 (F:B=3.68) **(S1-Figure 5f)**. Soil-Respiration C exhibited a 2rd order polynomial trend (r^2=0.97) (**S1-Figure 5f**) when regressed with initial soil F:B.

A further analysis of C partitioning as "*% of Total-System-New C partitioned in New-Soil C*", derived from dividing New-Soil C (g) by Total-System-New C (g) (**S1-Figure 6**), was conducted to assess the percent of Total-System-New C partitioned into New-Soil C mass (exudate, soil organic and microbial organic matter C mass). The Root C partition mass, which represented less than 14% of Total-System-New C, was not included in this analysis to isolate and identify only the net New-Soil C resources directed into the soil structure to support SMC growth and maintenance.

The "*% of Total-System-New C partitioned in New-Soil C*" in Treatment 0 (F:B = 0.04) was 93% of Total-System-New C, leaving only 7% of Total-System-New C partitioned into Shoot C and Fruit C (**S1-Figure 6**). As treatment Initial-Soil C along with its associated F:B increased, the "*% of Total-System-New C partitioned in New-Soil C*" exhibited a negative linear trend line (m = -0.12; r^2 = 0.94) to an end point of the six treatments where ~47% of Total-System-New C was partitioned into New-Soil C, and the balance, disregarding Root C, was diverted towards

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increasing C partitioning into Shoot C and Fruit C and a decreasing amount into Soil-Respiration C (**S1-Figure 6**).

3.1.3- Nitrogen Partitioning

Treatment N mass (g) was evaluated in six partitions for: Root N, Shoot N, Fruit N, Initial-Soil N, New-Soil N, and Total-System-New N, a summation of all new and/or replacement soil N in the preceding partitions. Treatment N mass was based on the average values derived from single-factor ANOVA analyses of plant and soil mass and N% data available in **(S1-Tables 1a-c, 2a-c, and 3a-b)**.

Flow of Total-System-New N into Root N, Shoot N and Fruit N and New-Soil N partitions is depicted in **Figure 2**. The mass of individual Shoot-N, Fruit-N, and Root-N exhibited positive linear regression trends (r^2 = *0.95, 0.97, and 0.83*, respectively) when regressed with soil F:B **(S1-Figure 7a, 7b, and 7c**). Collectively, Root N, Shoot N and Fruit N partitions exhibited a positive linear trend (r^2 = *0.97*) when regressed with soil F:B (**S1-Figure 7d**).

Partitioning of N mass (g) into the New-Soil N partition (**S1-Figure 7e**) was 0.2 g N in Treatment 0 (F:B=0.04) increasing to 0.88 g N up to Treatment 2 (F:B = 1.60), after which the New-Soil N production begins to decrease crossing the X-axis between Treatment 4 and Treatment 5, with a negative flow of -0.13 g N in Treatment 5 (F:B=3.68) **(Figure 2)**. New-Soil N mass followed a 3rd-order polynomial regression trendline (r^2 = 0.99) **(S1-Figure 7e**).

Nitrogen partitioning as a "*% Total-System-New N partitioned in New-Soil N*" demonstrated initial 98% of N flow into New-Soil N in treatment "0" (F:B=0.04). The *"% Total-*

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279 *System-New N partitioned in New-Soil N"* in Treatment "0" is significant but the mass of that N
280 partition was small (~0.2 g). In successive treatments (1-5), the "*% Total-System-New N*
281 *partitioned in New-Soil N*" diminished following a 3rd order polynomial trend line ($r^2 = 0.99$)
282 crossing 0% flow into New-Soil N, between Treatment 4 and Treatment 5, where eventually a
283 negative -32% percent, or a net flow of N from Initial-Soil N, was observed (**S1-Figure 8**).

284 **3.1.4- Soil Carbon Respiration**

285 Soil-Respiration C (g), estimated as the total respired C (g) over the 86-day growing
286 period in the 6 experimental soil treatments, demonstrated an increase from ~0.9 g C to ~4.3 g
287 C (**S1-Figure 5f**) from Treatment 0, plateauing into Treatments 4 & 5 following a 2nd order
288 polynomial trendline ($r^2 = 0.97$). This represents a 4.7 fold increase in Soil-Respiration C
289 occurring in treatment soils having: 19 times more available treatment soil C mass (2.05 to 38.6
290 g C); a more populous SMC with a 12% increase in bacterial mass (0.313 g to 0.352 g); 118 times
291 increase in fungal mass (0.011 g to 1.299 g) and a 92 times increase in F:B ratio (0.04 to 3.68)
292 (**Table 1**).

293 A "*Percent of Initial-Soil C Respired*" was derived by dividing Soil-Respiration C (g) by
294 Initial-Soil C mass for each of the treatments. The trend for Initial-Soil C respiration rates
295 decreased from approximately 44% of the Initial-Soil C (g) substrate respired in the Treatment
296 1 (F:B = 0.04) to approximately 11% of the Initial-Soil C (g) substrate respired in Treatment 5
297 (F:B = 3.68), (**Figure 3)**. This change in carbon respired over the range of treatment Initial-Soil C
298 mass and F:B, represented a 4-fold reduction in "*% of Initial-Soil C Respired*" and was best

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represented by a negative logarithmic trend line (m= -.108ln(x); $r^2 = 0.94$) when regressed with treatment Initial-Soil C (g) **(Figure 3**).

3.1.5- Total Carbon Use Efficiency

Treatment total carbon use efficiency (TCUE) is defined as net primary production (NPP), (total New-System C in Root C, Shoot C, Fruit C, New-Soil C partitions) divided by gross primary production (GPP) (New-System C plus Respiration C) using the formula: TCUE (%) = (NPP/GPP)*100 . This measure of the efficiency of C assimilation in this research does not include the maintenance costs of plant metabolic, anabolic or catabolic processes. The TCUE for Treatment 0, with no compost addition in treatment soil, exhibited a TCUE = 12%. All successive treatments (those with successive additions of compost) exhibited an average TCUE of 79%, varying over a range of TCUE values ranging from 75% to 84% (**Figure 4**).

3.1.6 Carbon Costs of N Fixation

The C costs of N fixation vary with host species, bacterial strain and plant, and estimates vary from between 12 g C/g N fixed to 6 g C/g N fixed (Streeter 1995; Vance & Heichel, 1991). The carbon costs for N fixation in each of the experimental soil treatments was derived using a conservative estimate of 6 g C/g N fixed, multiplying the mass of each N partition (Root N, Shoot N, Fruit N, and New-Soil N (g) fixed by a factor of "6" to get the cumulative total amount of C partitioned into new N fixation. This new partition was then added to the other 5 carbon partitions to yield "Total-Photosynthate C". Each C partition was then divided by this "Total-Photosynthate C" to derive what percentage of the "Total Photosynthate C "each of the other C

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partitions comprised and these percentages are displayed in the 6 pie-charts representing each of the six experimental soil treatments F:B (0-5) (**Figure 5**).

4.0- Discussion

4.1- Carbon Partitioning

The experimental design in this research was originally designed to have all initial treatment C and N mass, SMC, and F:B variables to exhibit linear increases. The purpose for this design was to help determine what treatment preconditions influenced C and N partitioning in this experiment. There are two potential causal-mechanisms for C and N partitioning in this experiment: **a)** simple nutrient/energy-resource availability from available elemental nutrients and carbon energy components from the added compost, and **b)** biological interactions and/or mechanisms involved in the "increases of" and "partitioning of" C and N mass into plant, soil and respiration partitions in this experiment.

The causal factors for the observed linear increases of C mass (g) in Root C, Shoot C, and Fruit C relative to Initial-Soil C (g), in this greenhouse experiment, could be partially explained from a nutrient/energy-resource availability perspective; where, the increasing concentrations of Initial-Soil C and its associated nutrient and energy-resource content in each treatment may promote corresponding linear increases in aboveground plant biomass. However, the cumulative mass of treatment Initial-Soil C + New-Soil C, and treatment Initial-Soil N + New-Soil N demonstrated increases in C and N mass in every treatment except for one, the final

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Treatment 5, where a reduction of -0.13 g soil N was observed in New-Soil N. The increases in C and N mass in all other treatments indicate that the Initial-Soil C and/or N mass, in all other observations, was either utilized and then replaced or was not utilized to begin with.

There are expectations that Initial-Soil C does have an influence on the observed partitioning of C into plant partitions; however it is difficult to design an experiment with increases in F:B accompanied by homogenous SMC populations without an increase in soil C. It is important to consider the properties of the flow of photosynthate-C into all partitions when conclusions are being made about the influence of Initial-Soil C on the flow of plant photosynthate into experimental treatment C and N partitions. Increases in Initial-Soil C do not explain: **1)** the immediate increase in system TCUE observed in Treatment 1 (**Figure 4**) when the inclusion of soil (compost) in the treatment soil fabrication mixtures promoted an immediate 6.5 times increase in TCUE, **2)** the partitioning of the "*% of Total-System-New C partitioned in New-Soil C*" following a negative linear trendline (**S1-Figure 6**), **3)** the reduced soil respiration rate following a negative log trendline with a 4-times reduction in the percent of Initial-Soil C respired(**Figure 3**); **4)** the controlled and coordinated flow of C into N fixation following a 3rd order polynomial trendline (**S1-Figure 7e**), and **5)** the decreasing influence of Initial-Soil C when correlated with Total-System-New C yielding a consistently diminishing slope in polynomial trend line (**S1-Figure 9).**

The trend lines of all of these observations (TCUE; New-Soil C, Soil-Respiration C, C flow into N-fixation, and reduced influence of Initial Soil C) do not follow a resource-availability hypothesis and are best correlated with 2nd and 3rd-order polynomial regression trend lines,

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indicating that mechanisms other than nutrient/resource availability are to be considered **(S1-Figures 5e & 5f, S1-Figure 7e).** The ebb and flow of the partitioning of C and N mass into New-Soil C & N and Soil-Respiration C partitions is most likely explained by the influence of treatment SMC population, structure, and biological functionality as mediated by plant/SMC interactions.

When considering only the flow of C into plant, soil and respiration partitions (**Figure 1)**, the maximum system photosynthate C productivity, occurred at a F:B of approximately 1.6:1, (Treatment 2) at which point Total-System-New C production plus replacement C for Soil-Respiration C reaches and maintains a maximum through the remainder of experimental treatments. Plant photosynthate, from Treatment 2 forward, is increasingly partitioned into plant Shoot C and Fruit C, with a lesser amount into Root C. This is demonstrated by continued linear increases of C into these plant biomass partitions in Treatments 3-5, with corresponding decreases in the amounts of photosynthate C partitioned into New-Soil C and Soil-Respiration C (**Figure 1**).

An interesting linearity is observed when considering the "% *of Total-System-New C partitioned in New-Soil C*" partition, **(S1-Figure 6)**. The results from this experiment reveal the ability of the plant/SMC supraorganism to preferentially partition up to 93% of plant photosynthate into low-fertility, low-carbon soil environments; and even in high fertility, high-carbon, high F:B soils, the plant/SMC supraorganism partitions 47% of plant photosynthate towards increasing New-Soil C. At first consideration, this allocation of plant photosynthate C toward the development of the components of New-Soil C would appear detrimental to the

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plant's survival, but other researchers theorize this may offer other benefits for the immediate and/or future development of SMC capabilities for supporting plant growth (Glick, 2012).

There have been many field observations that agricultural soils are 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 research appear to support observations of this threshold. Maximum Total-System-New C production is achieved by Treatment 2 (C%= 1.4, F:B ratio = 1.6), approximately 2.4% SOM. Photosynthate C, from this point forward, is increasingly partitioned into Shoot C and Fruit C, with decreasing amounts of photosynthate C directed towards increasing New-Soil C and New-Soil N (**Figure 1, and Figure 2**). Additionally, the apex of the curvilinear trend lines for New-Soil C and New-Soil N are both observed to reach a maximum at soil treatments of about 1.4% C, approximately 2.4% SOM, where there appears to be a satiation or tipping point, and the partitioning of both C and N mass into the soil environment begins to recede with a concomitant increase in the flow of photosynthate C into plant biomass. This observation may help explain the field observations in Loveland & Webb (2003) where soil is less productive when soil C percentages drop below 3.0% SOM (1.7% soil C). Based on the observations in the present study, the plant/SMC ecosystem appears to be capable of preferentially directing the flow of plant photosynthates toward improving either or both of the plant and/or soil C and N partitions toward mutually beneficial goals determined by the plant/SMC supraorganism.

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401 **4.2- Nitrogen Partitioning**

402 The C cost for nitrogen fixation varies with host species, bacterial strain, and plant
403 development. It is an energy-intensive process, and it requires considerable quantities of a
404 plant's photosynthate resources to accomplish (Gutschick, 1978). Estimates on C costs per gram
405 of N fixed vary from 12 g C (Streeter, 1985) to 6 g C (Vance and Heichel, 1991). This research
406 suggests there is a significant allocation of plant photosynthate energy resources towards free-
407 living or endophytic N-fixing bacteria, since chiles have not been observed to associate with
408 root-nodulating rhizobia. The increase in New-System N, resulting from growing chile, a
409 common commodity crop used in these greenhouse trials, is most likely increased through
410 beneficial interactions between chile plants and free-living or endophytic N-fixing soil bacteria.
411 The shifts in partitioning of "% *Total-New-System N partitioned in New-Soil N*", observed as
412 treatment F:B increases, demonstrates the potential for plant/SMC interactions to regulate the
413 flow of plant photosynthate C and/or Initial-Soil C energy resources toward diazotrophs for N
414 fixation.

415

416 **4.3- Soil C Respiration**

417 A 4.6-times increase in soil respiration rates (0.91 g C to 4.21 g C) (**S1-Figure 5f**) was
418 observed over a range of initial treatment soils conditions that demonstrated: a) 25-times
419 increase in Initial-Soil C mass (2.05 g C to 38.6 g C) (**Table 1**); and **b**) 5-times increase in SMC
420 microbial biomass (0.33 g to 1.65 g) (**Table 1**). Collectively, the observed Soil-Respiration C

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demonstrated a 4-fold reduction in "relative" soil C emission rates as a "*percent of treatment Initial-Soil C (g) substrate respired*". The reduction in Soil-Respiration C emission rates is potentially due to increases in SMC growth efficiency, potentially similar to the increase in bacterial growth efficiency (BGE) as observed by del Giorgio & Cole (1998) and Taylor & Townsend (2010), and also similar to the observations by Fontaine et al. (2011) where more fertile ecosystems had higher C assimilation rates. The reduction in relative soil respiration values from 44% to 11% of Initial-Soil C (**Figure 3**) characterizes the potential that higher fertility soils, defined as having SMC with fungal-dominated structures, have for improving both BGE and the retention of C compounds in soils, also observed by researchers in Six et al. (2006) and Kallenbach et al. (2016).

4.4- Total Carbon Use Efficiency

The TCUE values observed for each treatment in this experiment appear to be related to SMC structure and/or its biological functionality. The immediate increase of TCUE from 12% in Treatment 0 (no compost addition) to an average TCUE of 78% in all of the following Treatments 1-5 (**Figure 4**), (those with compost addition), indicates SMC structure and/or its biological functionality help determine the efficiency with which plant photosynthate is partitioned into secondary carbon structures of microbial organic matter and/or root exudates as opposed to respiration of that C.

5.0- Conclusions

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441 If we were to compare plant growth to F:B, and rely solely on observations for Shoot
442 and Fruit C and N partition mass, we would miss the influence of a SMC on critical C and N
443 partitioning mechanisms for plant-exudates into Soil C and N partitions, N fixation, Soil-
444 Respiration C and its related TCUE. Even though some researchers have observed that F:B may
445 be inconclusive, others are considering it as a potential biological metric to reliably estimate the
446 fertility of a soil. This research was designed to resolve this conflict by reducing the potential
447 interference that may result from SMC heterogeneity. The results observed in this experiment
448 indicate there is significant correlation between soil fertility, plant growth and homogenous
449 increases in the F:B of SMC with regards to the partitioning of plant photosynthate into Plant C
450 and N, New-Soil C and N, and Soil-Respiration C partitions. When observing the SMC-
451 dependent fluctuations of C and N partitioning in this experiment, it appears there is more
452 happening in the plant/SMC ecosystem than can be relayed by a simple measurement of F:B.
453 Assessment of microbial populations and structures (F:B), while proven informative by some
454 researchers, most likely provides a simple measure of soil fertility. This measure of SMC
455 structure will not relay the full potential and functional capability of a SMC to collectively
456 demonstrate population-dependent increases in functional diversity as in a quorum effect; or,
457 the potential for microbes to participate in interrelated or dynamic interactions between
458 multiple microbial components of SMC, and/or to characterize and display independent and/or
459 dependent interactions with plants in the plant/SMC supraorganism. An unexpected example of
460 this was observed in this experiment when, chile, a standard commodity crop that has not been
461 observed to associate with diazotrophs, demonstrated the ability to associate with free-living or

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462 endophytic N-fixing soil microbes and assist in partitioning C flow towards N-fixation in low-N
463 soil environments. Significant scientific resources are being invested to promote N-fixation
464 capabilities in commodity crops, but what may be missing is not a genetic component the plant
465 is lacking in its genome, but is actually the absence of a structurally or functionally beneficial
466 association with a healthy SMC population. Natural N-fixing mechanisms may be more likely to
467 occur when a complete "biologically functional" SMC is present. Promotion of robust SMC and
468 their biological functionalty, within the plant/SMC supraorganism, may help to reduce synthetic
469 N inputs deemed to be necessary in the SMC-compromised conventionally-managed soils of
470 our agroecosystems.

471 The ebb and flow of SMC-related C and N partitioning observed in this experiment may
472 offer important clues to biological mechanisms operating daily in the plant/soil foodwebs of
473 natural ecosystems. The results from this greenhouse experiment may give us a roadmap for
474 the transformations we may expect to observe as we promote the restoration of SMC F:B,
475 health and diversity towards improving soil fertility in agroecosystems. If these greenhouse
476 research results translate successfully into field applications, then application of agricultural
477 management approaches that enhance SMC population, structure and biological functionality
478 may promote: **a)** increased storage of photosynthate C and its partitioning into biomass (soil-,
479 plant-, and microbial organic matter; exudate production, etc.); **b)** increased storage of C in
480 soils, resulting from increased assimilation of SMC-C into microbial originated soil organic
481 matter; **c)** improved system TCUE, and reduce soil carbon respiration rates; and, **d)** increased

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assimilation of N using standard commodity crops through their association with free-living and/or endophytic N-fixing soil bacteria.

Enhancing SMC structure and biological functionality in soils of agroecosystems may be a logical and cost-effective path for improving crop production, reducing fertilizer inputs, and promoting a sustainable agricultural system while offering the potential to provide practical and cost-effective capture and storage of atmospheric carbon in soils of agroecosystems.

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599 **Figures and Tables**

600 **Table 1:** Initial treatment soil mass, soil C, soil N, and soil microbial community metrics.

601 **Figure 1:** Carbon partitioning (g) into New- Soil C, Root C, Shoot C, Fruit C, and Respiration C for
602 each treatment fungal:bacterial ratio.

603 **Figure 2:** Nitrogen partitioning (g) into New-Soil N, Root N, Shoot N and Fruit N for each
604 treatment fungal:bacterial ratio.

605 **Figure 3:** Treatment percent of Initial-Soil C respired (%) compared to Initial-Soil C content (g).

606 **Figure 4:** Comparison of total carbon use efficiency vs. treatment fungal:bacterial ratio.

607 **Figure 5**: Treatments 0-5 (F:B=0.14 to 3.68) percent of photosynthate C flow into plant (root,
608 shoot, fruit), soil, respiration and C consumption for N fixation.

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Supplementary Information

S1-Figure 1: Soil microbial community analyses of the compost used to mix soil treatments 1-5, 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 pre-experiment static alkali reactor sensitivity analyses, using data from 1–day, 2-day, and 3-day reactor operating times, conducted to confirm LOI reliability, CO_2 absorption characteristics, variance, and reproducibility with different reactor sampling intervals.

S1-Figure 4: Results from a GLM regression analysis, comparing initial calculated treatment soil mix (C%) with loss-on-ignition analyses (percent mass loss) to confirm experimental setup.

S1-Figure 5: Carbon partitioning (g) present in: a) shoot C, b) fruit C, c) root C, d) total plant C, e) new soil C, and f) respiration C as related to fungal:bacterial ratio.

S1-Figure 6: Percent of total system new carbon diverted to the soil as related to fungal:bacterial ratio.

S1-Figure 7: Nitrogen partitioning (g) present in: a) plant shoot N, b) fruit N, c) root N, d) total plant N, and e) total new soil N as related to fungal:bacterial ratio.

S1-Figure 8: Percent of Total-System-New N diverted to New-Soil N as related to

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639 fungal:bacterial ratio.

640 **S1-Figure 9:** Comparison of Initial C vs. Total-System-New C.

641 **S1-Table 1a-c**: Plant biomass data.

642 **S1-Table 2a-c**: Ending soil dry mass, soil carbon percent and soil nitrogen percent.

643 **S1-Table 3a-c**: Plant component nitrogen, carbon percent and cumulative soil respiration.

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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						
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

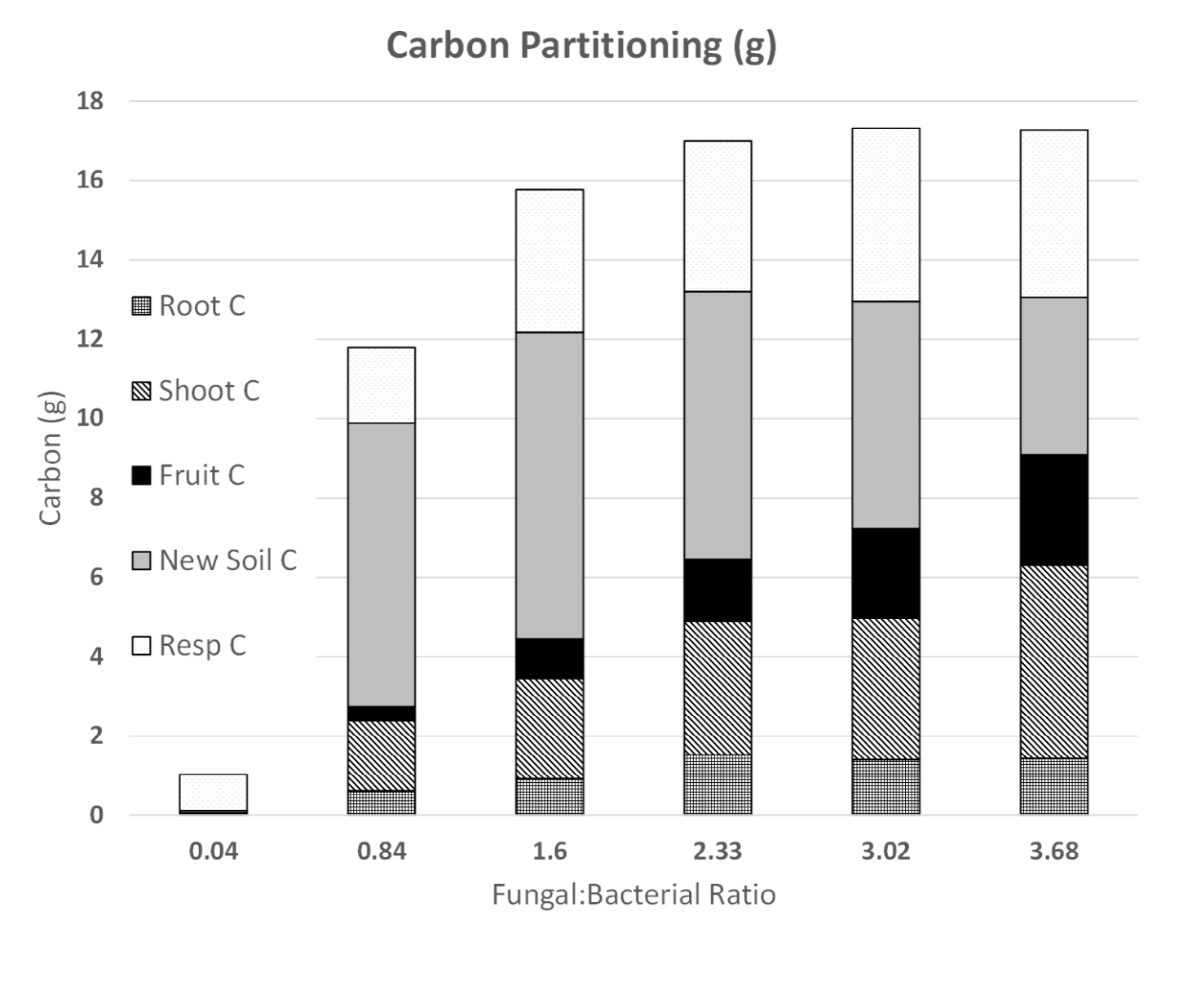

Figure 1: The stacked columns represent the carbon (C) partitioning (g) of New Soil C, Root C, Shoot C, Fruit C, Respiration C for each treatment Fungal:Bacterial Ratio (F:B) as designated by key.

Figure 2

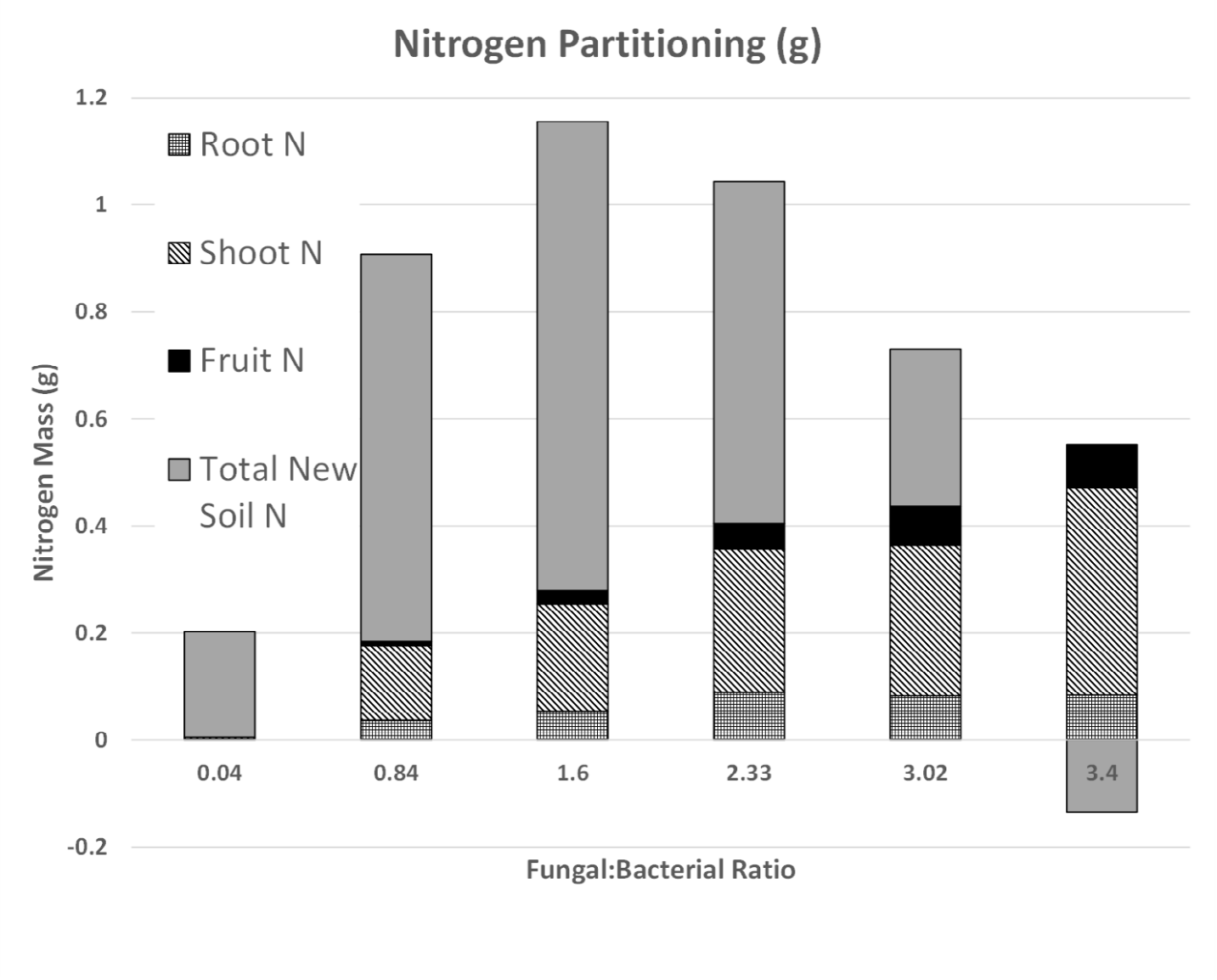

Figure 2– The stacked columns represent the nitrogen (N) partitioning (g) of New Soil N, Root N, Shoot N, Fruit N, for each treatment Fungal:Bacterial Ratio (F:B) as designated by key.

Figure 3

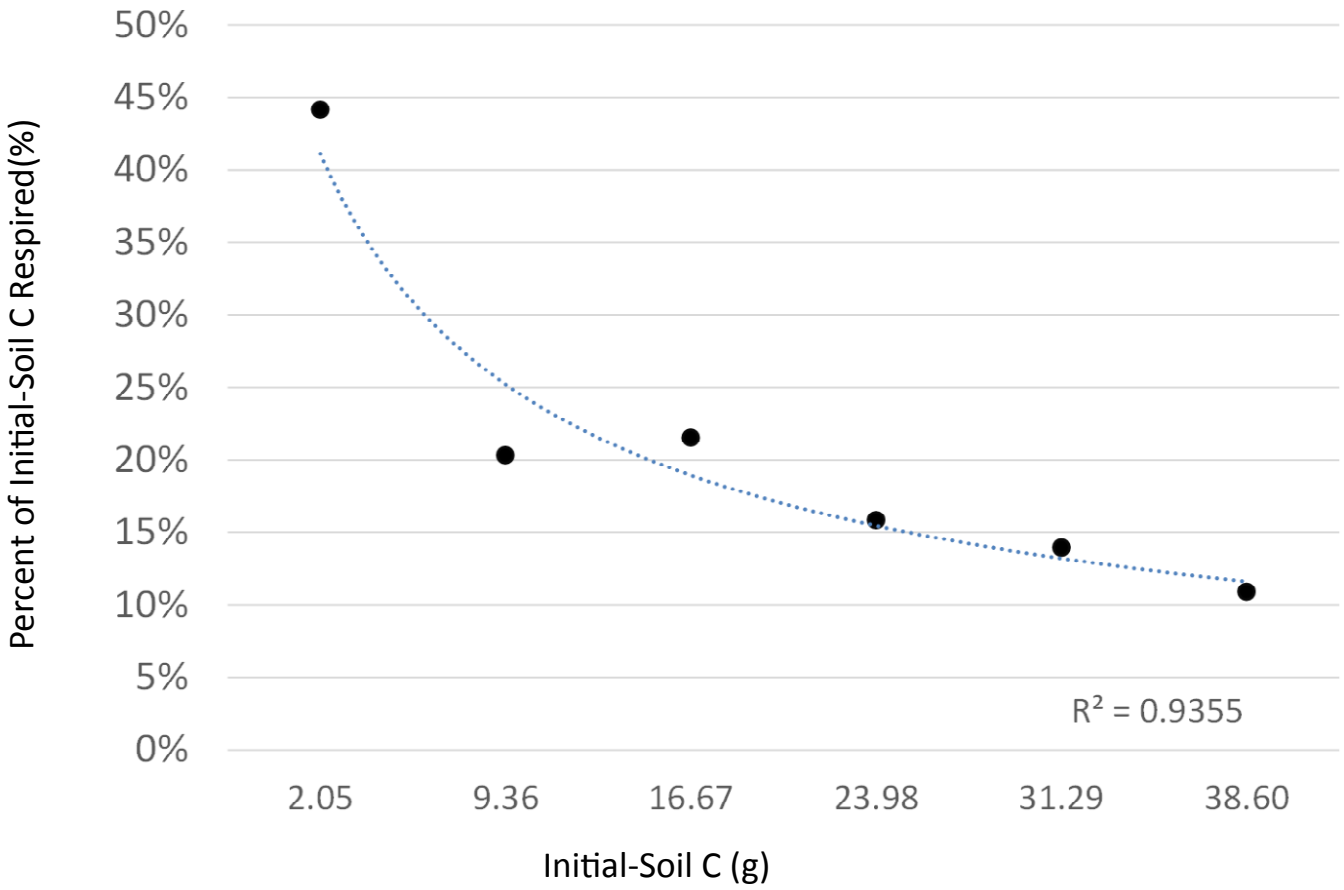

Figure 3: Treatment percent of Initial-Soil C respired (%) compared to Initial-Soil C content (g).

Figure 4

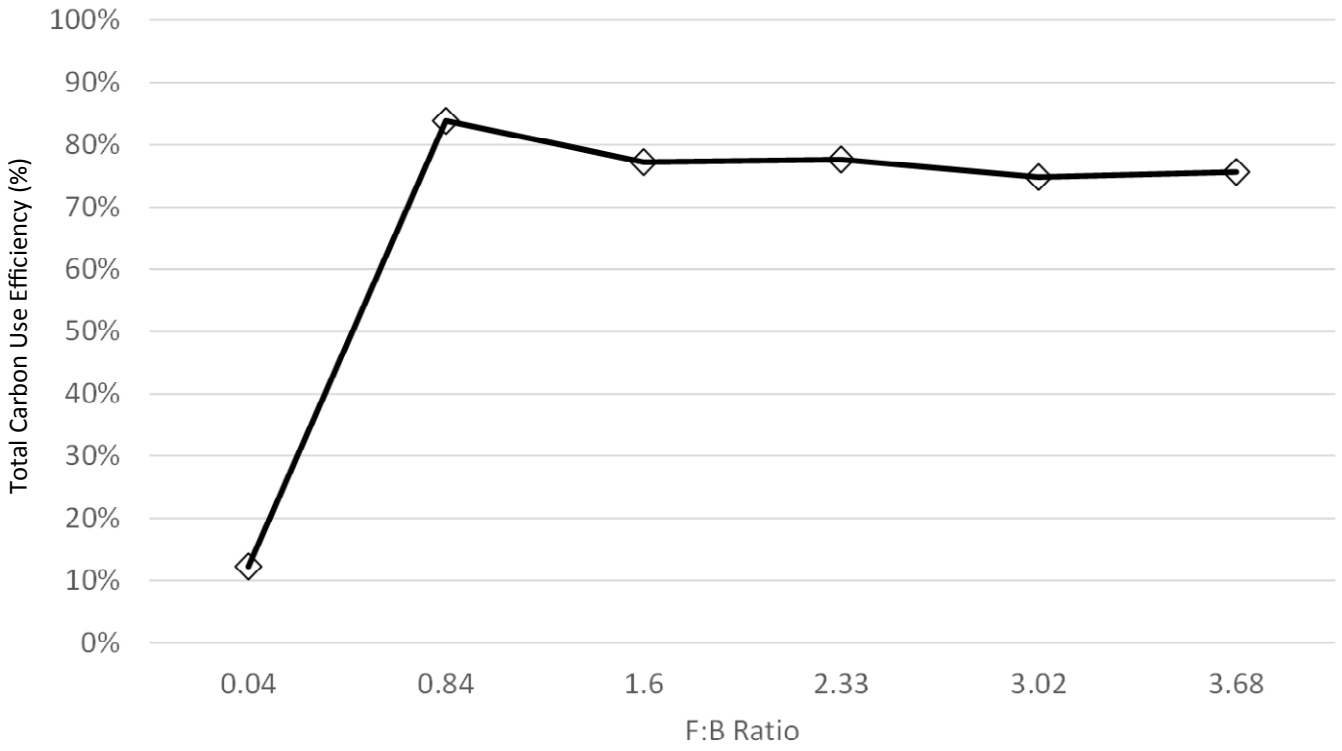

Figure 4: Comparison of total carbon use efficiency vs. treatment fungal:bacterial ratio.

Figure 5: Treatments 0-5 (F:B=0.14 to 3.68) percent of photosynthate C flow into plant (root, shoot, fruit), new-soil, respiration and C consumption for Total-New N fixation.