C:N Experiment

Introduction

What is Organic Matter?

One of the solid portions of the soil is called organic matter. Plant and animal residues in various stages of decomposition, monosaccharides, polysaccharides, amino acids, proteins, fats, waxes, and lignin that comprise cells and tissues, all are considered organic matter. Once the organic matter has been broken down it forms a dark brown and black unstructured and assorted substance referred to as humus. The main reasons for adding organic matter to soil are; to discard of any organic waste that is not useable, introduce nutrients needed for plants, and to prepare seed beds by increasing the tilth of the soil. The addition of organic matter has other benefits such as; better water holding capacity, improved aeration, decrease soil erosion, better soil structure, and increased cation capacity.

What is C:N?

The projected benefit of organic matter in an agricultural soil can be directly predicted and measured by the material's C:N ratio. The ratio of the mass of organic carbon to the mass of organic nitrogen is called the C:N ratio. The C:N ratio is inversely proportional to the nitrogen amount in soil. For example, a low (narrow) C:N ratio would have a high amount of nitrogen and a high (wide) C:N ratio would have low relative amounts of nitrogen. A straw for instance has a high C:N ratio and would be decomposed, or eaten and broken down, at lower rates than say legumes such as clovers or alfalfa with a low C:N ratio (high relative amount of nitrogen). This occurs because like humans, microorganism need energy in the form of nitrogen to carryout everyday tasks such as eating. When various life forms that come into contact with the amendments are given more nitrogen, they have more strength for reproducing, create more enzymes, and decompose organic matter more effectively. Conversely, if the C:N ratio is high, less nitrogen is available for microbes and the decomposition rate is reduced.

Carbon Cycle

The carbon cycle is a fundamental part of life on earth. The amount of carbon stored in the soil is a component of soil organic matter. Organic carbon in the soil is the basis of soil fertility. Carbon is a buffer against harmful pests, assists in releasing nutrients for plant growth, promotes improved structure, and enhances biological and physical health of soil. Organic material is manufactured by plants using carbon dioxide from the air and water. Plants and animals die and return to the soil where they are decomposed and recycled. Minerals are released into the soil and carbon dioxide is released into the atmosphere where it undergoes reduction reactions and gains electrons. It then returns to the soil where microbes strip CO_2 of its electrons in a process called oxidation thus completing the carbon cycle. The electrons are vital for producing enzymes that lower the activation energy requirements to decompose organic matter. Plants require carbon dioxide for photosynthesis and having more living things in the localized region will result in more carbon for crops.

Mineralization and Immobilization

The decomposition of organic matter by microorganisms is vital for plants. Nitrogen is needed but not all forms of nitrogen are bioavailable to plants. Microbes have an important task in this regard which is called mineralization where they consume free nitrates, create enzymes, and decompose organic nitrogen held within organic matter and transform it into inorganic plant bioavailable nitrogen. The process peaks at a rate that is affected by several environmental factors to include the nitrogen content. This comes at a temporary cost however, because the nitrogen needed by plants becomes temporarily unavailable when it is first absorbed into the tissues and cells of the organisms consuming the organic matter, termed immobilization. Since the organic matter lacks enough readily available nitrogen for microbes, they consume nitrogen in the soil to initiate the decomposition process of the organic matter. Immobilization creates a temporary nitrogen depression where plant growth is reduced. When the microbes consume all the nitrogen in the soil they die and then release nitrogen. This nitrogen then goes through a process called nitrification and ammonium ions are oxidized to nitrate ions. This whole process called the nitrogen cycle results in a net gain in total bioavailable nitrogen for plant consumption. If the organic matter has a low C:N ratio then the immobilization phase will be shorter and the net gain in inorganic nitrogen will be higher. On the contrary, the immobilization phase will be longer if the C:N ratio is higher resulting in a net gain in inorganic nitrogen but to a lesser extent of the former on a mass by mass basis.

Management of Nitrogen Immobilization

The management of nitrogen immobilization can be achieved by adding organic amendments with varying C:N ratios that correlate with desired goals and by managing conditions that are conducive for microbial health. Commonly used amendments include biosolids, animal manures, compost, cover crops, and crop residue. If adding nutrients immediately is desired, a material comparable to alfalfa is required because of its low C:N. Adding nutrients in subsequent growing seasons and improving soil structure and tilth can be accomplished using a high C:N material such as straw. Combined they can condition the soil thereby increasing quality and quantity of yields in both the short and long term. This process can be further assisted by addressing microbial health by maintaining a neutral pH, adding material when temperatures are between 70-100 °F if immediate decomposition is desired, sustaining moderate moisture, and generally fostering an ideal biological home for the organisms to live in.

Objectives of the experiment

Demonstrate the influence of organic matter’s C:N ratio on:

- Bioavailable inorganic nitrogen amounts
- Soil structure transformation and extent of enhanced tilth
- Microbial vigor in soil

Materials & Methods

Experimental set-up

Uniform amounts of the same sandy loam soil were place into experimental units (plastic boxes) and given different C:N treatments with various levels of replication. All 18 experimental units were initially treated with the equivalent of about 100 pounds nitrogen/acre-6 inches of calcium nitrate ($Ca(NO_3)_2$). Control boxes, to which nothing else was added, were replicated with a level of five. Five more experimental units received the addition of a cover crop legume mix consisting of a low 13:1 C:N ratio. The cereal cover crop with a medium 37:1 C:N ratio had a replication level of four. Lastly, a replication level of four was given to the high 80:1 C:N straw crop residue treatment. The experimental units were then covered with heavy black plastic, placed outside, and maintained at near uniform moisture levels. Although weather did vary over the 13 weeks from August 25, 2017 to November 17, 2017, the conditions were virtually unvaried for all experimental units with some negligible differences in temperature.

Quick Nitrate Soil Test Procedure

Every two weeks, soil was taken from six different locations within e given experimental unit and mixed. It was then placed into a cup containing 30 ml of a 0.01 M $CaCl_2$ extracting solution until volume reached 40 ml. It was shaken vigorously and filtered into a beaker until 75% of solution was collected. A nitrate-specific test strip was used to measure the filtrate by quickly dipping the strip into the filtrate and read precisely 60 seconds later. The color of the quick test strip was compared to the seven-color band on the box and the PPM of nitrate was determined. A dilution factor was utilized if levels were greater than 100 PPM.

Soil Respiration Procedure

Much like the quick nitrate soil test procedure, soil from six different locations was collected and mixed ensuring the moisture level was adequate and clods were removed. The 350 grams of soil was carefully added to a mason jar ensuring it did not come into contact with anyone's exhaled breathe. Then a plastic vial filled with sodium hydroxide (NaOH) was placed into the mason jar and covered for two weeks. As microbes decomposed the organic matter they emitted CO_2 which was absorbed by the sodium hydroxide. Each day the cap was lifted for one minute to introduce more oxygen ensuring microbial health. At the end of the two weeks, 5 ml of the sodium hydroxide was collected from the plastic vial and mixed with 20 ml of distilled water, 5 ml of barium chloride ($BaCl_2$) and six drops of indicator solution. Then a reverse titration was conducted with 1 M HCl to determine the amount of CO_2 emitted. Volume of titrate was subtracted from the "no-soil check" to obtain the ug CO_2/ grams of oven dried soil/ day. Less titrate implies more carbon dioxide evolved and thus correlated with more organic matter and its corresponding C:N ratio which resulted in more microbial activity.

Measurement of Soil Aggregate Stability

The combination of aggregation, structure, texture, bulk density, water holding capacity, workability, and infiltration is given the term tilth. The introduction of organic matter with various C:N ratios can enhance these properties. At the end of the 13 weeks a slaking or aggregate stability test was performed to monitor these qualities. Nine chunks of undisturbed soil measuring 6 mm X 2 mm each, were placed into round wire sieves and placed into deionized water to observe the soil's stability via its "melting" rate. If any soil remained on the sieve after five minutes a moderately paced dip test was performed five times. Based upon how much soil remained in the sieve it was given a ranking from 1-6 with 1 being the least amount of structural integrity and 6 having the most structural integrity.

Standard Error

Uncertainty and the degree of error in a reported measurement can be represented using error bars which are graphical representations of the variability of data. They provide a general indication of the accuracy of the reported values and give a quantitative value to the level of accuracy. Human error was held to a minimum and statistical noise was mitigated by replication and the use of averages. Outliers were also thrown out if human error was blatant. Within this context, error bars are still provided to ensure trends observed are within the normal limits of previously replicated experiments.

Results

Soil Nitrate

The replicated data (NO_3-N in ppm) from each of the four different treatments was averaged per nitrate quick test corresponding to the week in which it was conducted. *(Figure 1.1 & 1.2)* The first week, all four treatment averages had similar nitrate levels as expected. The legume mix had the fastest rate of increase in NO_3-N, peaking at week six. The nitrate levels rapidly declined but never fell below its starting value of 116 ppm of NO_3-N. The legume ended with a net gain of 33 ppm of NO_3-N. The straw treatment experienced the sharpest decline which occurred almost immediately. It then reached its lowest nitrate depression around week four with a slow but steady increase in nitrates. The straw saw a sharp decline in nitrate levels at week two and then began to increase in week 11 but was still far below its original nitrate amounts even at week 13. The straw within the snap shot of time never climbed above its preliminary nitrate concentrations although it was rapidly approaching a net increase in NO_3-N. The cereal treatment responded much like the straw, experiencing a nitrogen depression and elevation but at a faster pace. The cereal did not see a net gain in nitrate levels but was approaching its initial concentration and may have if the experiment was run longer. The control group was uneventful and remained nearly constant serving its experimental purpose even with some notable error.

Figure 1.1

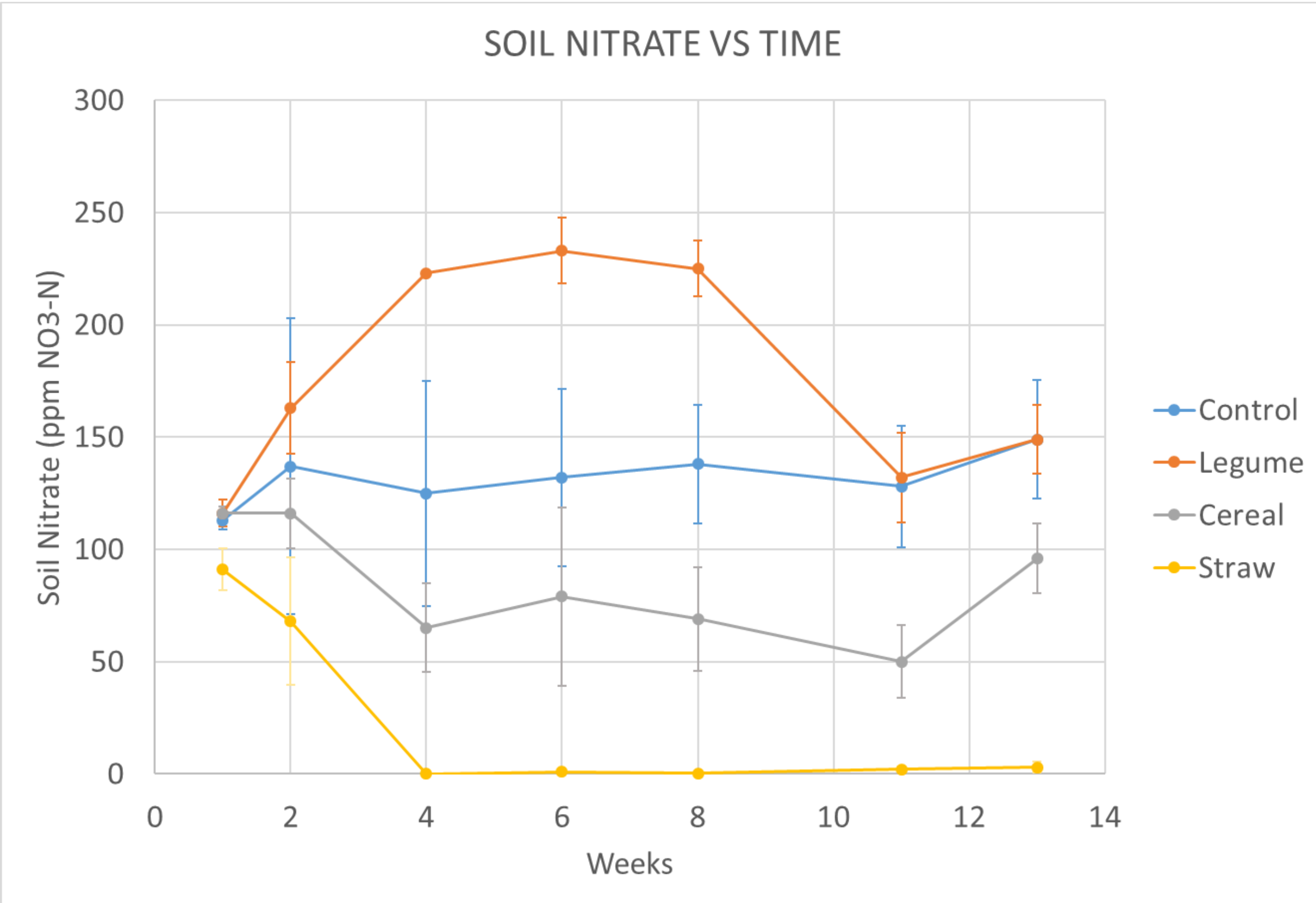

SOIL NITRATE VS TIME
Soil Nitrate (ppm NO3-N)
300
250
200
150
100
50
0
0
2
4
6
8
10
12
14
Weeks
Control
Legume
Cereal
Straw

Figure 1.2

C:N Soil Box Experiment

SW100L Fall 017

Soil nitrate quick test with visual reading, NO3-specific test strips

						Soil Nitrate (ppm NO_3-N)					
Treatment	***Rep***	***Initial Wk.1 (8/22)***	***Wk.2 (8/29)***	***nitrite***	***Wk.4 (9/12)***	***nitrite***	***Wk.6 (9/24)***	***nitrite***	***Wk.8 (10/10)***	***Wk.11 (10/31)***	***13 (11/14)***
Control	A	125	200		125		120		125	140	160
	B	112	125		125		100		125	63	125
	C	125	158		125		125		175	125	125
	D	109	125		125		188		.	188	188
	E	93	78		125		125		125	125	.
	mean=	***113***	***137***		***125***		***132***		***138***	***128***	***149***
	s.e.	*6.0*	*20.3*		*0.0*		*14.7*		*12.5*	*20.0*	*15.2*
Legume mix	A	113	75		90		150	++	300	67.5	80
(cover crop)	B	113	120		.	+	.		.	.	.
(C:N = 13:1)	C	125	60	+	200	+	300	++	200	125	137.5
	D	125	140		300	++	180	+	180	135	180
	E	105	420		300	++	300	+	220	200	200
	mean=	***116***	***163***		***223***		***233***		***225***	***132***	***149***
	s.e.	*3.9*	*65.9*		*50.1*		*39.4*		*26.3*	*27.1*	*26.5*
Cereal	A	125	75		75		113		75	75	125
(cover crop)	B	113	125		8	+	20	+	63	40	90
(C:N = 37:1)	C	113	113		100		175		125	75	113
	D	112.5	150		75		8		12	8	55
	mean=	***116***	***116***		***65***		***79***		***69***	***50***	***96***
	s.e.	*3.1*	*15.6*		*19.7*		*39.6*		*23.2*	*16.1*	*15.4*
Straw	A	113	35		0		1.5		0.5	0.5	0
(C:N = 80:1)	B	75	45		0		0.5		0.5	0.5	10
	C	75	.		0		0		0	5	1
	D	100	125		0		1.5		0.5	0.5	0.5
	mean=	***91***	***68***		***0***		***1***		***0.4***	***2***	***3***
	s.e.	*9*	*28*		*0*		*0.4*		*0.1*	*1.1*	*2*

** read off color chart, divided by 2 (correction factor: loam, moist soil)*

+ or ++ = formation of nitrite (NO2-) from nitirification following mineralization of the amendment. Can interfere (artificially increase the nitrate reading) thus any measured values > 300 were reduced to 300 as the higher readings are not valid. The nitrite formation as NH+ is converted to NO3- is short-lived.

Soil Respiration

The average amount of CO_2 that was evolved from each of the treatments measured in ug CO2/ grams of oven dried soil/ day in weeks 9-11. *(figure 2.1 & figure 2.2.)* The control group had the lowest amount of CO_2 trapped within the sodium hydroxide. Next was the legume followed by the cereal amendment. The straw had the most carbon dioxide emitted from the soil. The data corresponds to predictions of the C:N ratios of each ammendment. However, the standard deviation was quite high for the legume and cerel ammendments. The data ranged from 30-172 ug CO2/ grams of O.D. soil/ day for the legume treatment and 52-200 ug CO2/ grams of O.D. soil/ day for the cereal treatment.

Figure 2.1

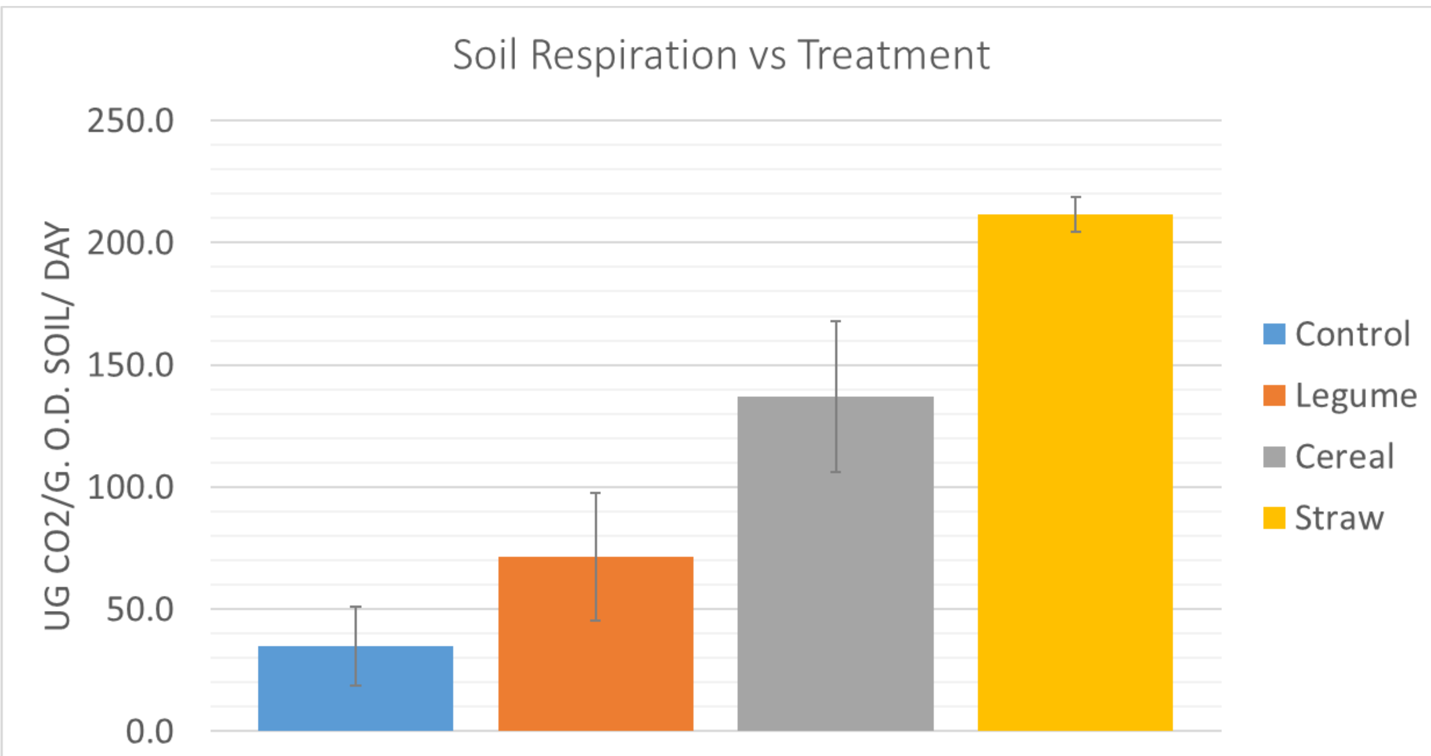


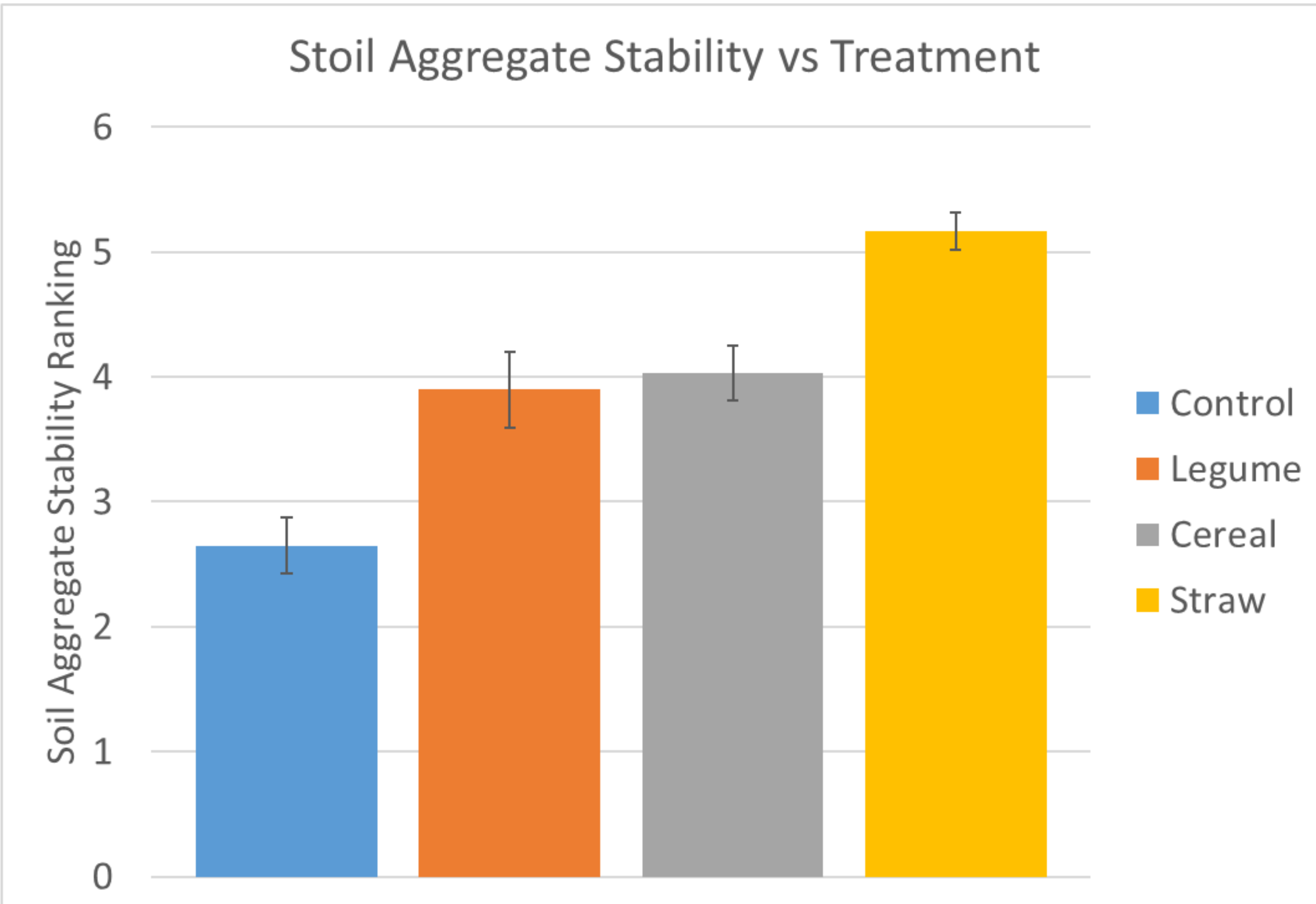
Figure 2.2

C:N Experiment 2017			SW100L
Assessment of Microbial Activity			
		C:N	*Soil Respiration*
Treatment		*(textbook value)*	*(ug CO_2/g. O.D. soil/ day)*
Control	A	--	2.59
	B		89.4
	C		38.0
	D		2.06
	E		43.2
	avg.		*35.1*
	S.E.**		*16.1*
Legume	A	low	69.2
cover crop	B	~13	172.6
	C		38.3
	D		30.3
	E		46.7
	avg.		*71.4*
	s.e.		*26.1*
Cereal	A	intermediate	156.6
cover crop	B	~37	137.8
	C		200.9
	D		52.7
	avg.		*137.0*
	s.e.		*31.1*
Straw	A	high	203.5
	B	~80	204.8
	C		205.3
	D		232.7
	avg.		*211.6*
	s.e.		*7.1*
***s.e. = standard error = standard deviation/ square root of N. N = # reps*			

Soil Tilth

As expected, the high C:N treatment of straw treatment had the best aggregate stability rating and the control group had the worst average stability ranking of all four treatments at the end of the 13 weeks. *(figure 3.1 & figure 3.2.)* In between and nearly identical in ranking, were the cereal and legume treatments.

Figure 3.1



Stability Rankings
1 = 50% of structural integrity lost, (melts) within 5 seconds of immersion in water
2 = 50% of structural integrity lost, (melts) 5–30 seconds after immersion
3 = 50% of structural integrity lost, (melts) 30–300 seconds after immersion OR (< 10% remains on the sieve after 5 dipping cycles.
4 = 10–25% of original soil material remains on the sieve after 5 dipping cycles
5 = 25–75% of original soil material remains on the sieve after 5 dipping cycles
6 = 75–100% of original soil material remains on the sieve after 5 dipping cycles
Note: higher number = greater aggregate stability (better soil structure)

Figure 3.2

C:N Experiment 2017 — **SW100L**

Assessment of Soil Tilth*

**Aggregate stability measured using a wet-sieving method.*
Higher number = greater stability (less slaking- loss of structure upon wetting)

			Soil Aggregate
		C:N	Stability Ranking
Treatment		(book)	(1 to 6)*
Control	A	--	3.07
	B		1.93
	C		2.95
	D		2.30
	E		3.00
	avg.		*2.65*
	s.e.		*0.23*
Legume	A	low	3.73
	B	~13	2.90
	C		4.68
	D		4.35
	E		3.82
	avg.		*3.90*
	s.e.		*0.30*
Cereal	A	intermediate	4.25
	B	~37	4.47
	C		3.47
	D		3.92
	avg.		*4.03*
	s.e.		*0.22*
Straw	A	high	5.45
	B	80	5.40
	C		4.95
	D		4.87
	avg.		*5.17*
	s.e.		*0.15*

***Only one straw box used for measurement, so no true replication. But there were 18 groups taking measurements on this box so we will call the 5.22 the "average". But we cannot calculate a standard error because only 1 soil box used for measurement*

Discussion

Soil Nitrate

In the first week, all four treatment averages had similar nitrate levels as expected because the microbes had not begun to decompose the organic matter and each treatment received uniform amounts of calcium nitrate (Ca(NO3)2). All experimental units received the initial nitrate treatment to provide energy for microbes to produce enzymes needed for the decomposition of their respective organic material treatment. The legume mix had the fastest mean rate of increase in NO3-N, peaking at week six. The nitrate levels rapidly declined but never fell below its starting point. Typically, an immobilization phase is witnessed but was drowned out by the nature of the averaged data within a narrow window of time. Two data sets did have an expected immobilization phase which was caused by microbes consuming all free nitrogen. The rapid increase then occurred because the high amount of nitrogen in the legume provided ample and readily available energy for microbe populations to rapidly grow, decompose the nitrogen rich amendments, and die. Nitrogen was mineralized from an organic to inorganic plant available form as the microbes died after nitrogen was consumed. The legume ended with a net gain of 33 ppm of NO3-N. The straw treatment experienced the sharpest decline which occurred almost immediately. It then reached its lowest nitrate depression around week four with a slow but steady increase in nitrates but was still far below its original nitrate amounts even at week 13. This was predicted because it is well known that high C:N ratios lack sufficient nitrogen for the decay of nitrogen deficient organic matter like a 80:1 straw. The steady yet very slight incline in nitrate within the straw treatment indicates microbes are beginning to die as the nitrogen in the straw is consumed and becomes unavailable. The control had some moderate error but the slight fluctuations were likely caused by the breakdown of some residual unknown organic matter already present in the soil. The cereal treatment essentially experienced the same phases of straw with the same rational but occurred at a more brisk pace because it had a lower C:N than the straw. Had data been collected daily for the first few weeks and extended beyond the 13 weeks, a clearer picture could have been achieved. Still, ample data exhibited that high C:N amendments are not desired if nitrogen is needed in the short term and conversely a low C:N is favorable if a crop requires liberal amounts of nitrate within the same growing season.

Soil Respiration

The straw released the most amount of carbon dioxide followed by cereal, legume and the control as expected. The straw having the least amount of nitrogen, did not provide enough enzyme production resulting in more energy needed to decompose the low N material and accordingly emitted more CO_2. Had this experiment been conducted in the very early stages, the legume may have had much higher amounts of CO_2 despite ample amounts of readily available nitrogen because individual microbes would have been competing with one another and the overall population would have dwarfed that of the straw due to advantageous microbial growing conditions. Despite some minor short comings of this assay's scheduling, the amount of CO_2 did offer a direct link to extent of microbial activity and provided more evidence to the level of energy needed by microbes to break down low C:N amendments. This assay can also provide growers with a moderately quantitative way of inferring the amount of organic matter already present in their soil and the soil's overall health.

Soil Tilth

Soil tilth or the physical health of the soil, can be measured several different ways but the basic slaking test was chosen for this experiment. Although the straw did not provide sufficient nitrates in the short term it did provide, yet again, direct evidence that high C:N amendments can improve the aggregate stability of soil as predicted. The microbes remained in the soil for longer periods of time because breaking down the straw was more difficult. While in the soil they built porous "mini-homes" and exuded bio-glues that hold the soil together creating a higher slake test ranking. The cereal crop with its higher C:N than the legume, provided slightly more improvements in tilth as expected. The control predictably had the worst ranking because microbes had little to feed on and thus did not multiply at a significant rate or discharge as much bio-adhesive sticky polysaccharides.

Conclusion

All objectives of the experiment were met with some degree of error but did nonetheless demonstrate the anticipated effects of organic matter, with various C:N ratios, on soil. Low C:N organic material like the legume, is excellent if vital plant available inorganic forms of nitrogen are needed within the same growing season and building structure of the soil is an ancillary goal. High C:N organic material, like the straw, while it provides less bioavailable nitrogen within the same growing season, can provide improved structure for the soil within the same growing season. Carbon dioxide being imperative for plants to carry out photosynthesis can be provided in elevated amounts if high C:N organic matter is utilized. Each growing situation is unique but all need nitrogen, carbon and a workable medium in which to grow. Different organic materials have unique benefits so it will likely require a combination of various organic materials with varying concentrations of carbon and nitrogen to achieve short term, intermediate, and long term goals.