Enlightened Soil Corp

En-Soil Algae

The Natural Path to Enhanced Soil Fertility

Two Reports: Haney Soil Test Results

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Two reports describing the effects of *C, vulgaris* on turf with a focus on Haney Testing.

Report 1

Effect of Live *Chlorella vulgaris* (En Soil Algae™) on Soil Microbial Activity (Soil Health): A Golf Course and Pasture Study

Kiawah Island, South Carolina
June-August, 2020
Dr. George Taylor
August 31, 2020

Introduction

Live algae as a soil amendment has been a subject of study by university agronomists for almost five decades, and farmers in lesser-developed countries have used it in place of more expensive chemical fertilizers. Multiple studies have shown enhanced growth of a wide variety of vegetables and grains as well as improved soil health, measured as soil organic matter. Live algae in micro-quantities produce compounds that stimulate bacterial growth in the rhizosphere, the neighborhood of the roots. These favorable microbes convert atmospheric nitrogen to organic compounds, nitrate, and ammonium that are used in plant metabolism.

We previously have field-tested *C.vulgaris* (En Soil Algae ™) with a variety of garden plants, and observed enhanced growth and vegetable production. These studies included leafy vegetables such as collard greens, kale, and lettuce.

In the present study we have extended these studies to turf with particular focus on the effect of live algae on soil composition and function using the Haney Soil Health Test (the primary outcome of the study). Like earlier studies, it measures organic matter, but it also evaluates other soil health indicators including soil respiration, and water-soluble fractions of organic carbon and organic nitrogen. Furthermore, it calculates a soil health score based on a combination of these different soil health indicators.

Methods

Golf Courses: Studies were conducted on the driving ranges of two Kiawah Island golf courses, The River Course (TRC) and Cassique (CSQ). The TRC test plot measured 2700 sq ft with a mixture of Seashore Paspalum and Bermuda 419 grass mowed to 1-1.5 inch height (the first cut of rough). The CSQ plot measured 2700 sq. ft., with Bermuda 419 grass closely mowed to fairway height, 0.5 inches.

Application schedule: the entire TRC and CSQ plots initially received three algae treatments at one-week intervals (a loading dose). To determine the optimal treatment interval, each of the 2700 sq. ft. plots was divided into three 900 sq ft sections and after the loading dose, the sections were treated 2 week , 4 week, or 8 week intervals.

Neglected Pasture: A third test site was a pasture that had not been fertilized for 20 years, and that was populated by wild grasses and weeds. After the three loading treatments, it was treated at 1-month intervals.

Soil Sampling: Each soil sample contained 1 cup soil (250 ml), a mixture of 5 augured cores taken to a depth of 4.5 inches (12 cm). The initial, control samples were obtained on June 9, 2020, before algae application. Follow-up sample from the three sections of the golf course plots were taken on August 10, 2020. In addition, there was a follow-up control sample taken from an untreated area at TRC, 30 feet from the test plot.

Algae Composition and Application: En Soil Algae ™, live *C. vulgaris* grown in sterile conditions, was obtained from the laboratory-production facility of Enlightened Soil Corp, Johns Island, SC (www.enlightenedsoil.com). The algae concentrate contained 10-12 million cells/ml. Five ml of the concentrate was diluted to a volume that treated 1000 sq. ft., or 50,000 cells per sq. ft. It was applied directly to the turf using a garden pump sprayer.

Results

The two golf course plots were mowed regularly, so there are no data comparing rate of growth. Grass in the pasture was not mowed, and from 4 to 8 weeks into the study grass in the algae treated area was 40% taller then adjacent untreated grass, and it was thicker as well.

Measures related to soil health were the primary outcome of this study, and are summarized in Table 1. At TRC there was there was an increase in soil organic matter (SOM), soil respiration, water extractible organic nitrogen (WEON), and water extractable organic carbon (WEOC). The index of soil health rose substantially.

At CSQ there was a similar improvement in soil respiration, WEON and the soil health index. There was less effect on WEOC. Of note, all measures of pretreatment soil health were higher at CSQ than TRC. The neglected pasture results paralleled those from the two golf courses (Table 1).

Treatment interval: At TRC there was an apparent dose response effect, with maximum improvement in soil health in the section treated at two-week intervals , and a decline in the effect with longer treatment intervals. That said, at both CSQ and TRC the soil health index was still above the control level with the two-month application schedule.

Table 1

Application Frequency	SOM	Soil Resp.	WEON	WEOC	Soil Health
TRC Control (06/09/2020)	2.3	55.8	4.4	79	7.61
TRC Control (8/09/2020)	1.7	52.9	13	101	8.61
TRC, 2 week intervals	5.5	173	25.7	138	19.76
TRC 4 week intervals	6.8	181	10.9	115	18.5
TRC 8 week intervals	3.2	77	6.6	110	10.59

CSQ Control 06/09/2020	4.2	84	7.5	124	11.62
CSQ, 2 week intervals	4.3	214	15.1	135	19.4
CSQ, 4 week intervals	2.8	101	12.7	87	11.4
CSQ, 8 week intervals	3.2	234	14.2	87	19.9
Pasture: Control	4.7	58	5.3	119	8.74
Pasture: 1 month intervals	5.5	113	11.7	87	12.30

Frequency: interval between applications after the 3-week loading period
TRC: The River Course, **CSQ:** Cassique Golf Course (the two courses of the Kiawah Island Club, Kiawah Island, SC)
SOM: soil organic matter, %LOI
Soil Resp: soil respiration: CO2-C in ppm;
WEON: water extractable organic nitrogen, ppm
WEOC: water extractable organic carbon, ppm
Soil Health Index: based upon soil respiration, WEON and WEOC
Discussion:

Live algae is unique among soil additives since it stimulates bacterial growth. Like chemical fertilizers, organic fertilizers (compost) provide nutrients, but have little effect on microbial activity. The bioactivity and biomass of this microbial community in the rhizosphere is synonymous with "soil health."

Previous studies have demonstrated that live algae application improves soil health measured as soil organic matter and number of bacterial species. They also documented increased plant yield (note the annotated references). The present results are consistent with those earlier studies.

We found that En Soil Algae ™ applied at 50,000 cells per sq. ft. resulted in improved measures of soil health using Haney Testing (a combination of tests introduced by Rick Haney, a USDA scientist). There has been some controversy about its utility for precisely calculating fertilizer needs for farmers; the argument is that the test is too new to relate it to outcomes on a large scale. For the purposes of this study there would be little controversy. Serial Haney Testing is considered reliable for showing trends in soil health following an intervention.

In both of the Kiawah Island golf course test plots as well as the neglected pasture there was a marked increase in soil respiration, a direct reflection of microbial activity. The Ward Laboratory Haney Test Interpretation Guide expresses it thusly: "Most microbes produce CO2 through aerobic respiration, just as we do, and the more CO2 a soil produces the more life it contains, or the higher the microbial biomass. This is important because it relates to a soil's potential for microbial activity, which is tied to many functions of a healthy soil such as nutrient cycling, soil aggregate and organic matter formation, disease suppression and stimulation of plant growth."

In like fashion, there was an increase in water extractible organic nitrogen (WEON) at both courses. Again from the Ward Laboratories Guide: "WEON represents the pool of organic N that is available to the microbes. Think of organic N as amino acids and proteins, which are linked to the carbon or food that the microbes are eating. "…Feeding the microbes an N rich food source allows them to better carry out many functions in the soil. One of these functions is N mineralization or the conversion of organic N into plant available forms such as nitrate and ammonium. In a healthy soil with greater biological function this can lead to a reduced need for synthetic N fertilizer."

The Haney soil analysis calculated that the TRC control (untreated) area had the equivalent of 15.3 lbs nitrogen per acre available "for the next crop", compared with 78.2 lbs N/acre in the section treated with algae at 2-week intervals. **This suggests that live algae could either replace nitrogen fertilizer or lower the amount required.**

The Ward Guide suggests that WEON is analogous to high protein nutrition for animals/humans, while organic carbon (WEOC) is like carbohydrates, a more easily metabolized source of energy. Plants, like animals, need both, but WEON would be considered a higher value food.

The result with organic carbon was less striking at CSQ than TRC. This could reflect a difference in mowing: grass in the TRC test plot was longer than that at CSQ (first cut of rough vs. tightly mowed fairway). Also, organic carbon is related to soil organic matter, and an important source of this is crop residue. In agriculture a common method to raise carbon input is a grass cover crop that is plowed in. However, a feature of golf course turf is that grass clippings are removed, reducing carbon input.

The present results help with decisions about algae application frequency. More frequent application led to increased benefit. That said, there was still a benefit when algae were applied at 8-week intervals. With these data, the following recommendation seem practical for treating turf:

1. Remember that there was a loading dose. This study applied algae weekly for three weeks to start the process. Application weekly x 2 or 3 could be considered at the beginning of the growing season.
2. With healthy grass, consider application at 6-week intervals during the growing season. More frequent application could promote more growth, but that means more mowing. We found a soil health benefit at even the 8-week application interval.
3. Trouble areas, bare places, can be treated more frequently.
4. Although not studied, freshly laid sod could benefit from more frequent application, every two weeks.
5. If the farming method inevitably affects soil quality—for example, sod growing where topsoil is part of the harvest—a two-week application schedule could prove a useful soil-building measure.

Measuring plant growth is the obvious approach to assessing efficacy of any soil amendment. The present results support serial Haney testing to follow soil microbial activity and biomass, e.g. soil health.

A final consideration is that the present study is limited to two months follow-up. The study is ongoing, and 6-12 month results will provide additional insight into efficacy and application frequency with chronic use of *C. vulgaris* as a soil amendment.

Report 2

Turf Trials of the Effects of *C. vulgaris* as a Soil Amendment: Fall 2020 Studies

January 22, 2021

This report will provide Haney test results for the following truf trials

1. 4-month follow-up from The River Course (TRC), and Cassique (CSQ) driving ranges
2. 4-month follow-up from Cougar Point
3. Results from a Johns Island horse farm growing winter ryegrass
4. Results from a study of golf course fairways at TRC that were growing winter ryegrass.

TRC and CSQ driving ranges: 4-month results (Table 1)

Table 1 presents data gathered before algae application (June 25, 2020), and four months later (November 2). The initial report (Report Number 1) described the Haney results two months after the beginning of the study. The test plots were near the back of the driving ranges on both courses and measured 2700 sq. ft. Each was divided into three sections. One section was treated at 2-week intervals, another at 4-week intervals, and the third, 8-week intervals. Results at varying application intervals appeared in Report Number 1. The present summary includes the 4-week and 8-week application interval results, since applications at this frequency are practical for turf (e.g. during the growing season turf is fertilized at 4-8 week intervals).

During the study the driving ranges, including the two test plots, were treated with regularly scheduled application of a synthetic chemical fertilizer (NPK). That is to say, this is a comparison of NPK + Algae vs. NPK alone.

Table 1. Haney test results comparing pretreatment control s(06-29-2021), and after 4 months of treatment (11-02-2021) with NPK alone or NPK + algae applied at 4 week intervals, or NPK + algae applied at 8 wk intervals. There were two applications of NPK during the 4-month study period.

	pH	SOM Health	Soil Resp	WEON	WEOC	Soil
TRC control (NPK, no algae)	7.3	2.3	56	4.4	79	7.61
TRC—4 mos. Algae + NPK Application interval = 4 wks	5.9	6.8	181	10.9	115	18.5
TRC—4 mos. Algae + NPK App. Interval = 8 wks	4.6	3.2	77	6.6	110	10.6
CSQ control (NPK, no algae)	7.5	4.2	84	7.5	124	11.6
CSQ—4 mos. Algae + NPK App. Interval = 4 wks	7.5	4.3	148	15.0	174	17.3
CSQ—4 mos. Algae + NPK App. Interval = 8 wks	7.7	3.8	95	13.8	156	14.1

SOM, soil organic matter (%LOI), Soil Respiration (CO_2-C, ppm C), WEON, water extracted organic nitrogen (ppm N), WEOC, total organic carbon (ppm C), Soil Health, an index derived from other measures, with weighting of soil respiration.

Cougar Point Driving Range (Table 2)

The Cougar Point Golf Course (Kiawah Island) has not been treated with synthetic fertilizers for more than 10 years. It is on a regular schedule of organic inputs with the exception of the body of the driving range, which is amended only if there is difficulty with growth. The body of the range had received no treatment for the year before this study.

The body of the 8-acre driving range was divided, and half was treated with algae. Algae were applied two weeks in a row, then at 4-week intervals. The follow-up soil samples reported in Table 2 were obtained three months after the initial treatment, and two weeks after the most recent treatment with algae. Thus, there were 4 applications of algae before the follow-up soil test.

In addition to the body of the driving range, the hitting area at the proximal end of the range (mown fairway close) was included in the trial. As noted, the body of the range received only algae as an amendment during the study. The hitting area received organic inputs on the golf course's usual schedule, plus algae.

Table 2. Effect of algae treatment on 2 sections of driving range, including the body of the range ("Range" with no previous soil amendments), and the short-grass hitting area ("Hitting area" with scheduled organic inputs).

	pH	SOM Health	Soil Resp	WEON	WEOC	Soil
Cougar Pt. Range (08-05-2020) Control, pretreatment	7.6	1.8	27	7.6	62	4.7
Cougar Pt. Range (11-04-2021) After 3 months treatment	7.0	2.8	115	13.8	157	14.1
Cougar Pt: Hitting area control, pretreatment	8.3	1.4	21	7.4	60	4.0

Cougar Pt: Hitting area After 3 months' treatment	7.4	3.0	96	14.3	149	14.0

Horse Farm (Table 3)

The purpose of this study was to observe the effect of algae on the growth of winter ryegrass. The farm is located on Johns Island, SC. There were 3 fenced pastures that were treated, two of them with algae and no additional input. The third pasture—Pasture 3—has been a problem area with a history of poor growth of both the perennial grass and winter rye. This was divided, and half was treated with NPK, the other half with chlorella vulgaris (En-Soil Algae) alone. Neither of the other pastures—Pastures 1 and 2—received NPK.

The farm's perennial grass is bahia grass (Paspalum notatum). Winter ryegrass was planted the first week of November, 2020. Haney soil testing was done just before, and repeated on 12-29-2020, seven weeks after seeding. Algae were applied 2 weeks in a row (before and just after seeding), then at 6-week intervals. Thus, it was applied three times during the study.

(Full Table on Next Page)

Table 3: Effect of *C. vulgaris* on soil health in horse pastures recently planted with annual ryegrass. Soil tests were done before and 7 weeks after seeding.

	pH	SOM Health	Soil Resp	WEON	WEOC	Soil
Pasture 1 (pretreatment) (a.k.a., the front pasture)	6.8	3.0	62	12.9	163	10.71
Pasture 1 (at 7 weeks)	6.7	4.5	191	12.5	119	19.6
Pasture 2(pretreatment) (the back pasture)	6.2	3.0	28	12.9	179	7.6
Pasture 2 (at 7 weeks)	5.7	2.8	48	8.0	184	9.27
Pasture 3, the problem pasture: half was treated with NPK, half with algae						
Pasture 3—pretreatment (NPK half)	7.1	4.1	89	12.0	154	13.2
Pasture 3—NPK alone (at 7 weeks)	6.7	3.0	59	10.5	190	10.8
Pasture 3—pretreatment (Algae half)	6.9	3.5	46	12.3	171	9.2
Pasture 3—Algae alone (at 7 weeks)	6.6	3.5	154	11.4	108	16.1

The effect of algae on soil respiration and the soil health index were similar to those noted in the golf course trials. However, on the horse farm we did not see a rise in organic nitrogen and organic carbon. Possible explanations are 1) there was active growth of ryegrass, and microbial utilization of N and C was at a high level, and/or 2) this trial was done at a change in season, with the follow-up soil testing in cold weather, on December 29. The four months of the golf course study (Table 1) ended with soil samples on November 2, still warm weather in coastal South Carolina (although at that time perennial grass was beginning to go dormant, so is consuming less N and C)

Additional trial at The River Course (Table 4)

This study was conducted on three parallel golf holes at TRC, numbers 14, 15 and 16. Numbers 14 and 15 were treated with algae two weeks in a row as well as a single application of NPK. Number 16 was treated with NPK alone (serving as a control). Unlike the driving range trials in the summer, this fall study included seeding and growth of winter ryegrass. The two algae applications were done just after seeding. Soil samples were collected before seeding, September 28, 2020, and the follow-up samples on November 29 (7 weeks after the first algae application, 6 weeks after the second).

(Full Table on Next Page)

Table 4. Effect on *C. vulgaris* as a soil amendment on Haney testing results, Holes 14, 15 and 16 at TRC.

	pH	SOM Health	Soil Resp.	WEON	WEOC	Soil
TRC No. 16 (Control, NPK alone)	7.3	2.6	39	15.4	160	8.64
TRC 16, at 7 weeks	7.5	2.6	41	6.6	157	7.9
TRC No.14 (NPK + Alage), control	7.7	2.3	33	15.6	106	7.0
TRC 14, at 7 weeks	7.4	2.7	69	8.1	172	11.1
TRC No. 15 (NPK + Algae), control	7.0	2.2	43	13.1	88	7.4
TRC 15, at 7 weeks	6.9	2.5	64	9.4	160	10.5

Like the horse farm study, there was a rise in soil respiration and the soil health index, but a decline in organic nitrogen (although there was a rise in organic carbon). Again, the follow-up soil samples were done during rapid growth of the winter ryegrass.

Comments

Four different turf trials, each with a wrinkle.

There are a lot of data in these tables, and there is some variability (making them more believable). The most consistent finding is that *C. vulgaris* application boosted soil respiration and the soil health index (which is calculated using soil respiration as well as WEON and WEOC). Soil respiration is the most direct measure of microbial activity; *C. vulgaris* has been shown to generate compounds that enhance microbial growth and activity, so the present results are consistent with published work.

The utility of the extended driving range trials at TRC and CSQ (Table 1) is just that: results extended to 4 months, through most of the growing season of perennial grass. The results are consistent with those of 2-month follow-up soil testing: organic matter, soil respiration, organic nitrogen and carbon, and the soil health index continued to be higher than the pretreatment control values.

The studies at CSQ and TRC were done on turf that continued to be treated with synthetic chemical fertilizer, NPK. On the other hand, the Johns Island horse farm study included a head to head comparison of NPK (alone) vs. Chlorella (alone), and the Cougar Point study was of algae (alone), without NPK. NPK has been shown to promote growth of turf (yes, it works). However published studies have shown that improved growth with NPK is not accompanied by a change in organic matter, soil respiration or organic carbon, all measures of microbial activity (aka, soil health). That is consistent with our findings at the Johns Island horse farm: NPK promoted growth, but did not affect the Haney test measures of soil health. In like fashion, the later TRC study (Table 4), found no change in the Haney analysis on hole number 16—treated with NPK alone. However, improvement was noted when algae was added (holes 14 and 15, Table 4).

An unexpected finding was that organic nitrogen did not consistently rise in the studies that involved rapidly growing winter ryegrass. In the other studies of turf in a stable growth phase, the rise in organic nitrogen was a consistent finding. The most plausible explanation is that with active new growth of ryegrass, there is increased N consumption (compared with steady state growth). Since the ryegrass studies were done at a change in season—the emergence of colder weather—that cannot be excluded as another explanation. A change in seasons/temperature adds a variable that may be best avoided in future studies.

We do not report the effects of chlorella on growth of turf in this report. Our previous report described increased growth in another test plot of a neglected meadow on Johns Island. Reported here, a side-by-side, qualitative comparison on the

horse farm, Pasture 3, indicated similar growth with NPK (alone) and algae (alone). And there are ample published documenting the growth promoting effects of algae as a soil amendment. Instead, this report has focused on the effect of live algae application on soil health/microbial activity. This approach is consistent with the growing conviction that we need to pay attention to farming the soil; *C. vulgaris* (En-Soil Algae) can contribute to that process.