Use of Enterra Natural Fertilizer (Black Soldier Fly Larvae Digestate) As a Soil Amendment

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Executive Summary

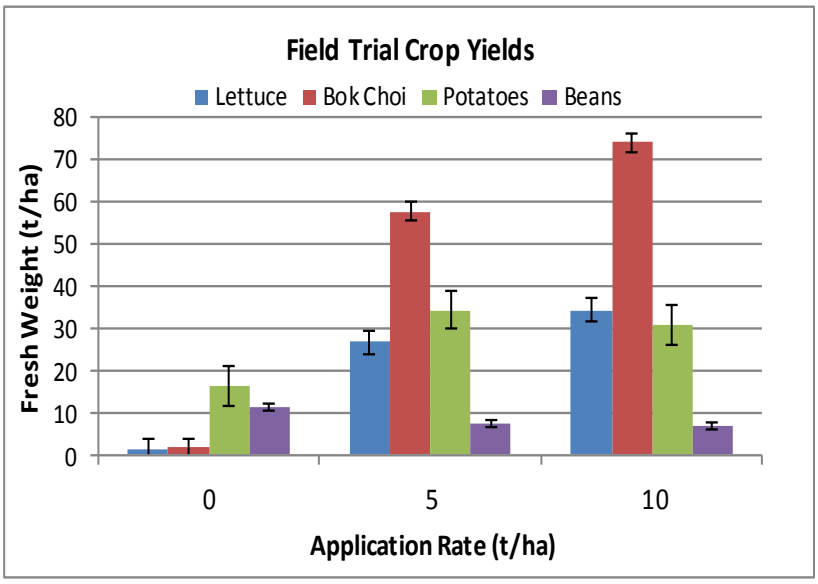
Certified Organic produce growers face a constant challenge in procuring reliable, sustainable sources of soil nutrients for intensive crop production. Enterra Feed Corp has developed a bioconversion process using the larvae of black soldier fly (BSF, *Hermetia illucens*) to convert food waste into a high-value animal feed. A co-product of the bioconversion process is Enterra Natural Fertilizer (ENF), the digestate of the larvae. This project, comprising three trials, evaluated plant nutritional value to establish recommendations for use of ENF in field and containerized applications.

Incubation Trial: To establish the soil nutrient and mineralization profiles of ENF compared to commercially available organic fertilizers like composted poultry litter (CPL) and worm castings (WC).

	Fertilizer NPK Analysis (%) DW Basis			% Available of Total		
Fertilizer Type	Total N	Available P_2O_5	Soluble K_2O	Total N	Available P_2O_5	Soluble K_2O
ENF	4.66	2.40	3.00	34	85	100
CPL	4.19	1.80	2.90	30	43	100
WC	2.81	0.05	0.05	4	16	17

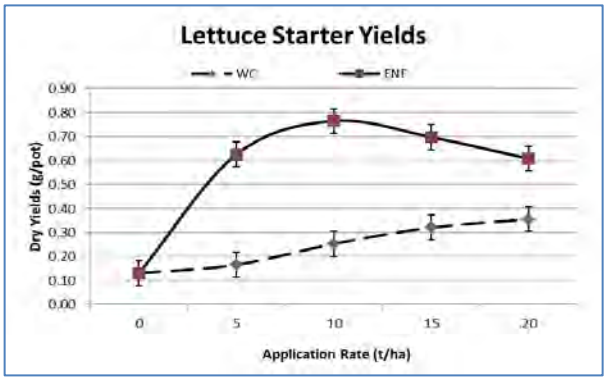
The ENF incubation results demonstrate high concentrations of N, P and K relative to other organic fertilizer sources. The rapid mineralization and early availability of nutrients of ENF are indicative of its favorable C:N ratio. Relative to the control, each t/ha of ENF raised soil EC by 0.2 dS/m, whereas the increase in EC associated with the composted poultry litter was 0.1 dS/m.

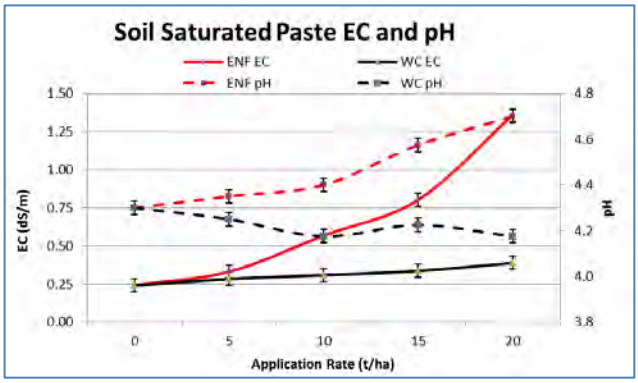
Field Trial: To establish the efficacy of ENF in supporting commercial yields of four crops in the field at low, medium and high application levels.



ENF increased crop yields for bok choi, lettuce and potatoes. Fertilizer effects on yield were confounded by mortality; however, surviving plants at the 5 and 10 t/ha application rates showed increased yields. Differences between the 5 and 10 t/ha rates were minimal. Although there were no significant differences in wireworm populations among treated plots, ENF may have provided wireworm protection (under further investigation).

Starter Trial: To establish the optimal ENF application rate for production of starters of six commercial vegetable crops and compare with worm castings, and to characterize toxicity, if any, at high application levels.



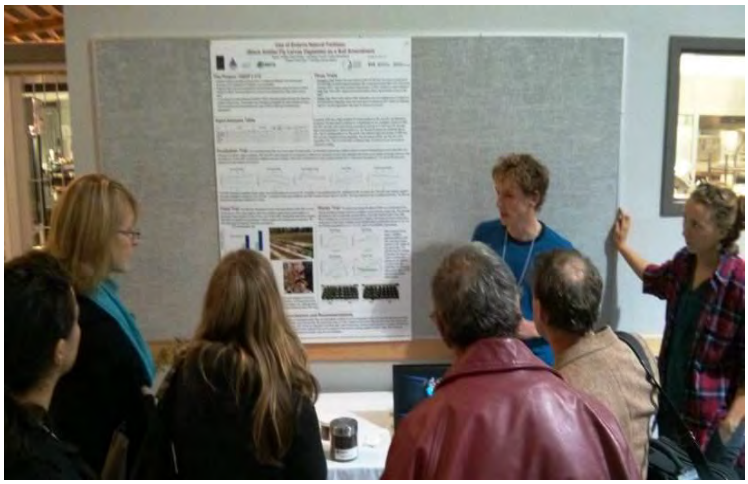


Lettuce Starters: ENF on right, WC on left, control in centre.

ENF produced larger and healthier/greener crops than worm castings applied at the same rates. The optimal ENF application for biomass yield for lettuce and squash was 5.3% DW/DW (10 t/ha), while 8% (15 t/ha) was optimal for bok choi, tomato, bean, and onion. EC increased with greater ENF applications but remained below critical levels. The cause of declining biomass at ENF pplications above 10 t/ha is unknown.

Conclusions and Recommendations

Enterra Natural Fertilizer is a well balanced fertilizer comparable to composted poultry litter, but with higher available N and exchangeable K and a narrow C/N ratio that promotes rapid mineralization when applied to soil. In the relatively low fertility soil at the test site, field application rates of 5 t/ha supports commercial yields of, bok choi, lettuce and potatoes. It also may have the potential to protect against wireworm. However more research is needed to establish a direct effect and investigate possible mechanisms. Inclusion of Enterra Natural Fertilizer at 5 to 8% DW/DW in peat/perlite starter plant media supports plant growth superior to that of worm castings applied at similar rates. More trials are required to determine optimum use and application method, rate and timing for specific crops and soil conditions.



2013 UFV Field-to-Fork event. Chilliwack. BC.

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Introduction

Enterra Feed Corporation (formerly Industrious Nature) received an OSDP grant to partially fund nutritional studies of Enterra Natural Fertilizer (ENF) produced from Black Soldier Fly larvae digestate. The goal of the project was to collect information to aid in fertilizer registration and formulation of recommendations for the use of this novel fertilizer in organic agriculture.

Three interlocking studies were designed to examine nutrient dynamics in relatively low fertility soil at a test site on Vancouver Island. The centrepiece of the project was an **incubation trial** – detailed documentation of nutrient release and mineralization over time that compared ENF to two commonly used organic fertilizers, composted poultry litter (CPL) and worm castings (WC). A **field trial** tested yield responses to two application rates of ENF on 4 crops planted in field plots. A third study – the **starter trial** – compared ENF and worm castings at increasing rates as fertilizers in the production of starter plants.

The product analysis of ENF positions it well in comparison to the two other organic fertilizer sources (Table 1). The most evident attribute is its high nitrogen content: approximately 60% more nitrogen than either the composted poultry litter or worm castings. In the organic agriculture industry, nitrogen availability is often a significant limiting factor to production.

Phosphorus and potassium concentrations are also high in Enterra Natural Fertilizer (Table 1), making it a great source of the three most commonly limiting nutrients. Lower concentrations of calcium and magnesium are not significant drawbacks as they are infrequently limiting, and easily met with other sources. The only potentially limiting attribute is the moderately high EC and sodium content.

From a physical perspective, the higher density of the ENF as compared to composted poultry litter provides an advantage in handling, transportation and ease of application.

Table 1: Macronutrients, micronutrients, and selected physical and chemical characteristics of the fertilizers used.

Fertilizer Inputs	Total (%)							
	C	N	S	P	K	Ca	Mg	Na
Enterra Natural Fertilizer	42.9	4.54	0.49	1.23	2.44	0.64	0.13	1.67
Worm Castings	38.8	2.57	1.78	0.14	0.25	3.45	0.30	0.09
Composted Poultry Litter	40.7	2.80	0.65	1.81	2.24	3.69	0.66	0.40
Standard Error	0.6	0.04	0.02	0.03	0.02	0.15	0.01	0.01

Fertilizer Inputs	Total (mg/kg)				
	Cu	Zn	Fe	Mn	B
Enterra Natural Fertilizer	11	49	471	13	7
Worm Castings	22	42	15231	520	22
Composted Poultry Litter	91	669	1775	718	31
Standard Error	0.7	3	204	7	0.6

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Fertilizer Inputs	Bulk Density (g/cm^3)	LOI (%)	Saturated pH	EC (dS/m)	C/N Ratio	Available (mg/kg) NH_4-N	NO_3-N
Enterra Natural Fertilizer	0.49	88	5.5	44	9	9675	538
Worm Castings	0.50	74	5.0	7	15	1485	1097
Composted Poultry Litter	0.23	79	7.3	11	15	2600	182
Standard Error	0.01	2	0.04	1	0.5	197	22

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Methods

Incubation Trial

Experimental Design:
Three fertilizers were selected for comparison, all at a rate equivalent to 10 t/ha by dry weight, as well as a control (no fertilizer) and ammonium nitrate applied at 100 kg/ha equivalent to act as an analytical and performance standard:

1. Control: no fertilizer
2. Enterra Natural Fertilizer (ENF; Enterra Feed Corp.)
3. Worm Castings (WC; Nurturing Nature Brand Vermicompost)
4. Composted Poultry Litter (CPL; Thomas Reid Farms, Langley)
5. Ammonium Nitrate (Sigma)

Plastic tubs (500 ml) with lids were used as incubation containers. Each had four 6 mm holes punched at intervals just below the rim to provide ventilation. Soil from the field trial site was limed with $CaCO_3$ to pH 6.5 using the buffered pH lime requirement, then sieved through a 5 mm screen. Prior to application of treatments, soil was allowed to equilibrate for a period of 2 weeks. Each tub received 300 g of soil mixed separately with the appropriate amendment prior to loading to minimize variability among the 20 replicates per treatment. Soils were compressed to a bulk density of 1 g/ml after loading in the tubs. Ammonium nitrate was added in 10 ml aliquots, so 10 ml of water was also added to all other treatments. Tubs were then randomized and placed into insulated totes and kept between 18 and 20 ^{O}C for the duration of the experiment. Four randomly selected replicate tubs of each treatment were taken for analysis at the following intervals:

1. T_7: sampled 7 days after incorporation of fertilizers
2. T_{14}: sampled 14 days after incorporation of fertilizers
3. T_{28}: sampled 28 days after incorporation of fertilizers
4. T_{56}: sampled 56 days after incorporation of fertilizers

Soil from tubs removed from incubation at each date were air dried for one week before they were ground, bagged and sent to Pacific Soil Analysis Inc. (PSAI, Richmond, BC) for analysis (for example, Table 2).

Table 2: Characteristics of limed soil used in the incubation trial prior to treatment application (T_0). (*n*=4).

		bulk density	Leco			2:1	Sat. EC	Available N kg/ha			Bray P	Exchangeable kg/ha				Ca:Mg	K:Mg	CEC
Soil Use		g/cm^3	%C	%N	%S	pH	dS/m	NH_4-N	NO_3-N	TAN	kg/ha	Na	K	Ca	Mg	Ratio	Ratio	meq/100g
Incubation Trial	Mean	0.96	4.84	0.32	0.043	5.3	0.68	28	66	94	28	83	101	1913	103	19	0.98	29.7
(Loam)	SD	0.02	0.10	0.01	0.002	0.0	0.04	2	1	2	4	12	9	256	3	3	0.06	0.7

The soil used in the incubation trial was a loam. Its CEC value was high but its base saturation was low at 25% (data not shown). Organic matter (%C) was high; available N, pH and salinity levels were low; available P and exchangeable bases (K, Ca, Mg) were also low, indicating that fertilizer inputs to sustain intensive vegetable crop production were necessary. The Ca:Mg and K:Mg ratios both suggest that Mg fertilizer inputs are required.

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Field Trial

Site

The field trial was sited at Amara Farm in Courtenay, Comox Valley, BC. The soil is valley bottom, fine textured and semi-poorly drained with a high water holding capacity; and is classified as a Humic Gleysol. The site, which had been in pasture, was plowed in March. Lime ($CaCO_3$) requirement was calculated to raise the soil pH to 6.5 and was applied at 10 t/ha.

Experimental Design

For the first planting, three levels of ENF were applied: control (0 t/ha), low (5 t/ha), and high (10 t/ha). All applications were made on a dry weight basis. A complete randomized block design (*n*=4) was used for each crop (Figure 1). For the second planting, lettuce and bok choi plots were split in half with one half of each plot receiving a 5 t/ha application of ENF and the other receiving no reapplication of ENF.

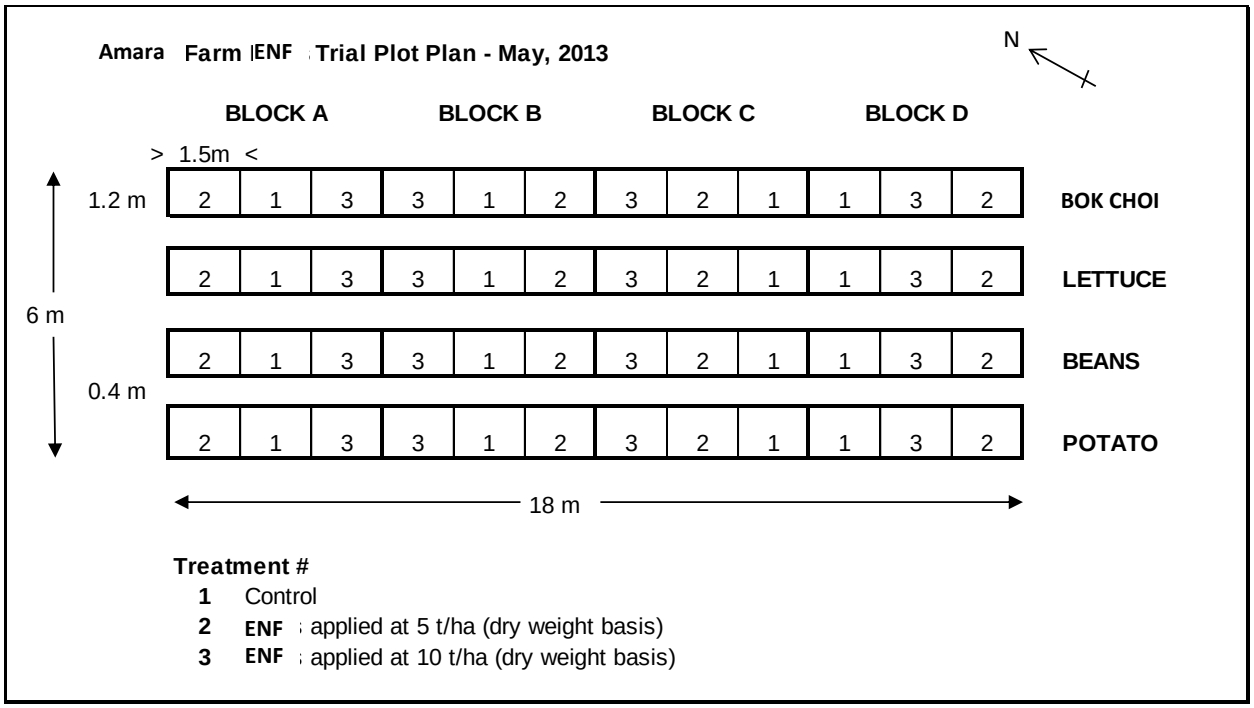

Figure 1: Field Trial plot plan for planting 1.

The treatments for planting 1 were applied on June 5th and incorporated to a depth of 15 cm. Plots were then leveled with rakes, covered with jute coffee sacks and left for 2 weeks. After the 2 week period the sacks were removed in preparation for planting.

Crops were planted as detailed In Table 3. Lettuce and Bok choi seedlings were started in a peat/perlite potting medium enriched with alfalfa meal, bone meal, and greensand. Plots were hand weeded throughout the course of the experiments. Irrigation was applied when water tension readings exceeded 25 cbar and drip irrigated until the soil water potential reached 10 cbars.

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Table 3: Field Trial crop planting log

Crop	Date Planted	Planting Characteristics
Potatoes cv. Warba	June 19th	20 cm deep Single row down middle 30 cm between tubers
Beans cv. Venture Blue Lake	July 11th	4 cm deep 2 rows 40 cm apart 7.5 cm between seeds
Lettuce, planting 1 cv. Flashy Trout Back	June 19th	Transplanted 3 rows 30 cm apart 30 cm between plants
Bok choi, planting 1 cv. Joi Choi	June 26th	Transplanted 3 rows 30 cm apart 40 cm between plants
Lettuce, planting 2 cv. Flashy Trout Back	August 21st	Each plot split in 2 across row; 9 plants per sub-plot in a 3x3 square w/ ~20 cm between plants
Bok Choi, planting 2 cv. Joi Choi	August 21st	Same as Lettuce, planting 2

Sampling and Analysis

Data were collected from the field plots in several categories: plant growth and development (marketable yield, stand characteristics, plant health), plant tissue analysis (nutrient concentrations and uptakes), and soil analysis (basic soil fertility characteristics).

Growth and Development

Marketable yield and/or above ground biomass of bok choi, lettuce, and beans was determined by harvesting and weighing plant biomass in each plot, then calculating yield per unit area. Potato marketable yield was nil for all plots due to excessive wireworm damage, however total tuber biomass was recorded.

Dry weight yields for bok choi, lettuce, and beans were estimated from moisture contents derived from grab samples of the marketable harvest. Samples were dried at 70 ^{o}C for 72 h.

For lettuce and bok choi, mortality was recorded at intervals from planting through harvest.

Diagnostic Tissue Testing

Bok choi: diagnostic samples were taken according to the Nova Scotia Agriculture worksheet "Vegetable Crop Tissue Sampling Guide" (Nova Scotia Agriculture, 2010) for cabbage because no bok choi diagnostic sampling guidelines were available. In addition, a whole-plant sample was taken at harvest to determine crop nutrient uptake for use in a nutrient budget.

Lettuce, beans, and potatoes: diagnostic samples were taken according to the Nova Scotia Agriculture worksheet "Vegetable Crop Tissue Sampling Guide" (Nova Scotia Agriculture, 2010). Sample values were compared to the diagnostic values provided in the worksheet.

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Soil Analysis

Plot soils were sampled (0 – 15 cm depth) at planting and at harvest to determine available and residual soil nutrients. Table 4 presents the soil characteristics analyzed for the field trial prior to planting.

Table 4: Site soil characteristics before lime or fertilizer addition (2 weeks prior to first planting). (*n*=4).

		bulk density	Leco			2:1	Sat. EC	Available N kg/ha			Bray P	Exchangeable kg/ha				Ca:Mg	K:Mg	CEC
Soil Use		g/cm^3	%C	%N	%S	pH	dS/m	NH_4-N	NO_3-N	TAN	kg/ha	Na	K	Ca	Mg	Ratio	Ratio	meq/100g
Field Trial	Mean	0.78	4.99	0.36	0.049	5.4	0.55	45	29	73	38	71	162	2456	144	17	1.13	35.3
(Loam)	SD	0.02	0.25	0.02	0.005	0.1	0.07	4	5	7	5	4	9	72	12	2	0.04	3.2

Starter Trial

Six crops in four replicates for each treatment level were planted to assess the effect of the fertilizers on a wide variety of crops. A peat and perlite (3:1 by dry weight) starting medium (Table 5) was made and mixed with ENF or worm castings at 5 different application rates: 0, 5, 10, 15, and 20 t/ha equivalent. Fertilizer was mixed individually into each replicate at each treatment level to reduce variability among replicates. Bok choi (var. "Joi Choi", West Coast Seeds), lettuce (var. "Flashy Trout Back", Johnny's Selected Seeds), snap beans (var. "Venture Blue Lake", West Coast Seeds), tomatoes (var. "Gardener's Delight", West Coast Seeds), squash (var. "Delicata", West Coast Seeds) and green onions (var. Kimcho, West Coast Seeds) were planted in 4 inch pots according to seed packet directions and maintained at just below field capacity for the duration of the study.

Table 5: Characteristics of the potting medium used in the starter trial. (*n*=4).

		bulk density			2:1	Sat. EC	Available N kg/ha			Bray P_1	Exchangeable kg/ha				Ca:Mg	K:Mg
Soil Use		g/cm^3	%N	%S	pH	dS/m	NH_4-N	NO_3-N	TAN	kg/ha	Na	K	Ca	Mg	Ratio	Ratio
Starter Trial	Mean	0.16	0.64	0.16	4.2	0.28	264	6	270	14	135	228	5393	968	5.6	0.24
(Peat)	SD	0.003	0.07	0.01	0.0	0.03	10	6	14	6	4	25	187	45	0.2	0.02

Statistical Analysis

All data were subjected to analysis of variance (ANOVA). Significance was set at $p<0.05$. Mean separation tests were performed on significant ANOVA *F*-tests.

Incubation Trial

The incubation trial was the core of this suite of experiments. It was intended to generate a reliable model of nutrient mineralization rates for the ENF product and compare it to other similar organic fertilizers. Worm castings and composted poultry manure were selected for comparison as similar organic fertilizer products; ammonium nitrate was selected for its known mineralization characteristics.

An accurate model of nutrient mineralization is especially important for organic fertilizer sources with high carbon contents and variable proportions of other nutrients such as ENF, worm castings, and animal manures. The chemical and biological complexity of these fertilizers means their chemical nutrient analyses do not reliably reflect the availability of nutrients and the subsequent impact on the crop.

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In order to determine appropriate application rates of organic fertilizers, it is necessary to determine the rates at which nutrients become available, the forms in which they are present, as well as the total available. This allows recommendations for fertilizer use that effectively meet the changing nutrient demands of a crop over the growing season, thereby economizing on fertilizer use and nutrient losses.

Results

ENF provided the nearly twice the level of available soil nitrogen across the time span of the incubation trial compared with composted poultry litter. The release of nitrogen from ENF was rapid with much of it becoming available by day 14. By day 56 available nitrogen level from ENF was still increasing, whereas the nitrogen levels of the other fertilizers had leveled off (Figure 2). The percentage of total N that was available by day 56 for the ENF, composted poultry litter, and worm castings was 34%, 30%, and 4%, respectively (see Table 6). Worm castings provided very little available nitrogen.

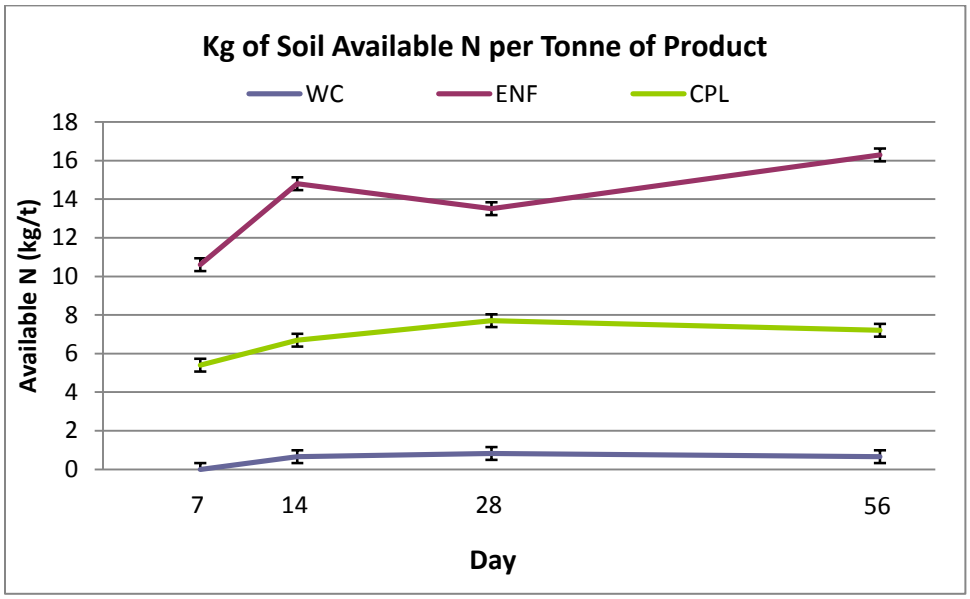

Figure 2: Kg soil available N per tonne of product applied over time.

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ENF provided the second largest amount of phosphorous over the study period after composted poultry litter which had a higher initial P analysis (Figure 3). All treatments exhibited a reduction in available P over time as it became immobilized in the soil.

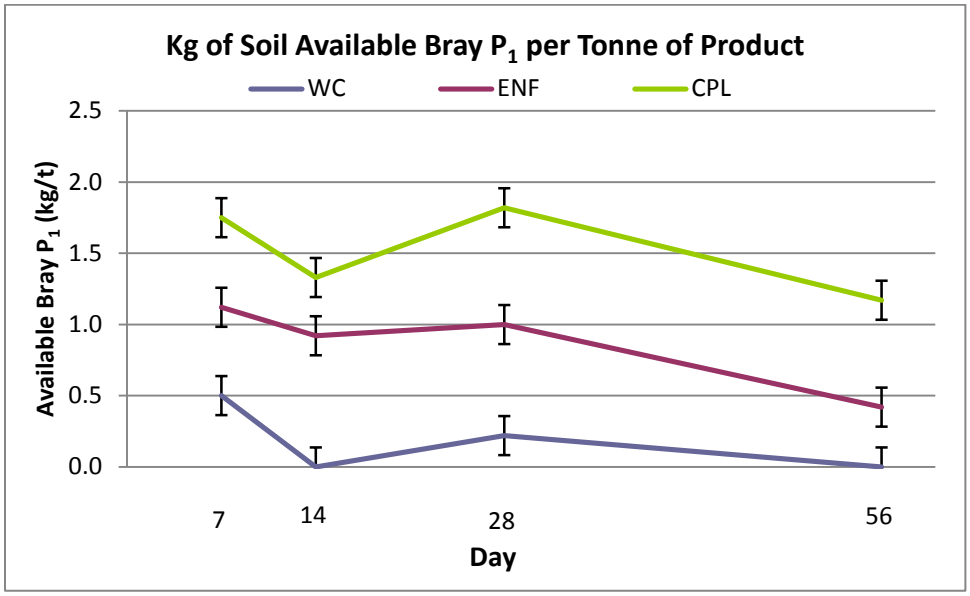

Figure 3: Total Soil Available Bray P_1 phosphorous over time. Error bars represent standard error.

ENF contributed the most potassium to the soil of the fertilizers studied, although the amount narrowed to within the range of the composted poultry litter by day 56 (Figure 4). These results suggest that the exchangeable K becomes available soon after application.

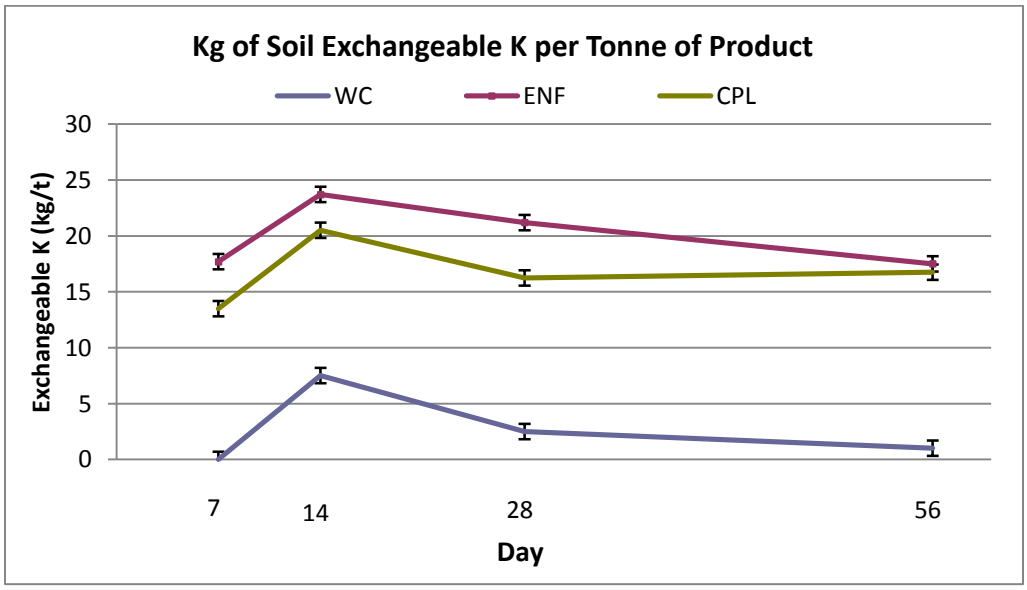

Figure 4: Soil exchangeable potassium over time. Error bars represent standard error.

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ENF has a relatively high EC, reflecting higher salt content than the other fertilizers. ENF produced EC levels twice those of composted poultry litter (Figure 5). Relative to the control, one t/ha of ENF raised soil EC by 0.2 dS/m, whereas the increase in EC associated with the composted poultry litter was 0.1 dS/m; that of the worm castings was negligible.

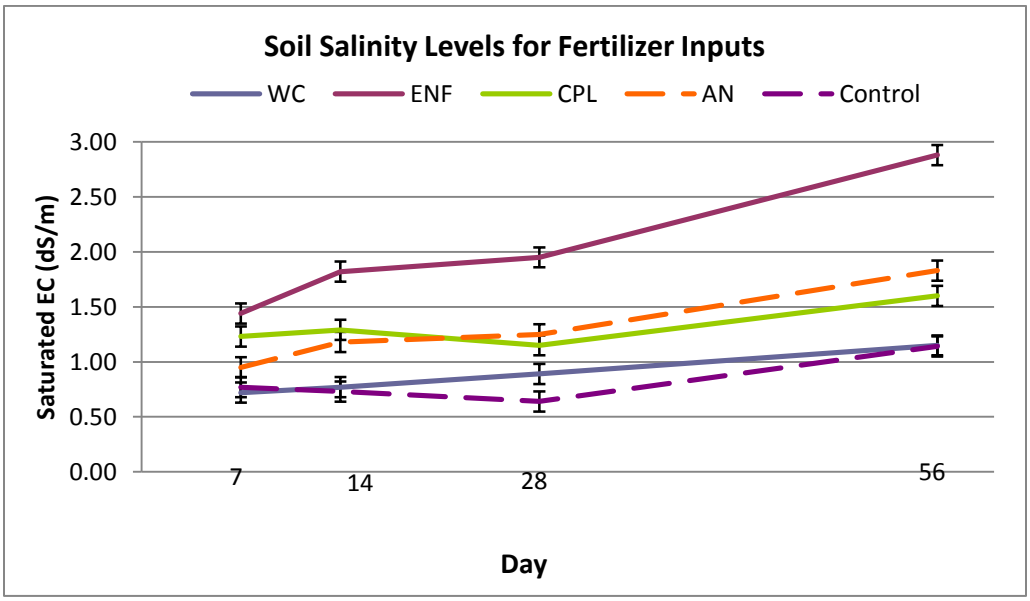

Figure 5: Soil salinity (electrical conductivity) resulting from the various fertilizers over time. Error bars represent standard error

ENF contributed substantially more sodium to the soil than the other fertilizers (Figure 6). The higher salinity levels associated with ENF appear to be related to its high sodium content. Sodium levels in ENF fertilizer may be reduced by reducing high-sodium inputs in the production process.

There appears to have been some sodium immobilization over time, as was observed with other exchangeable ions and phosphorous.

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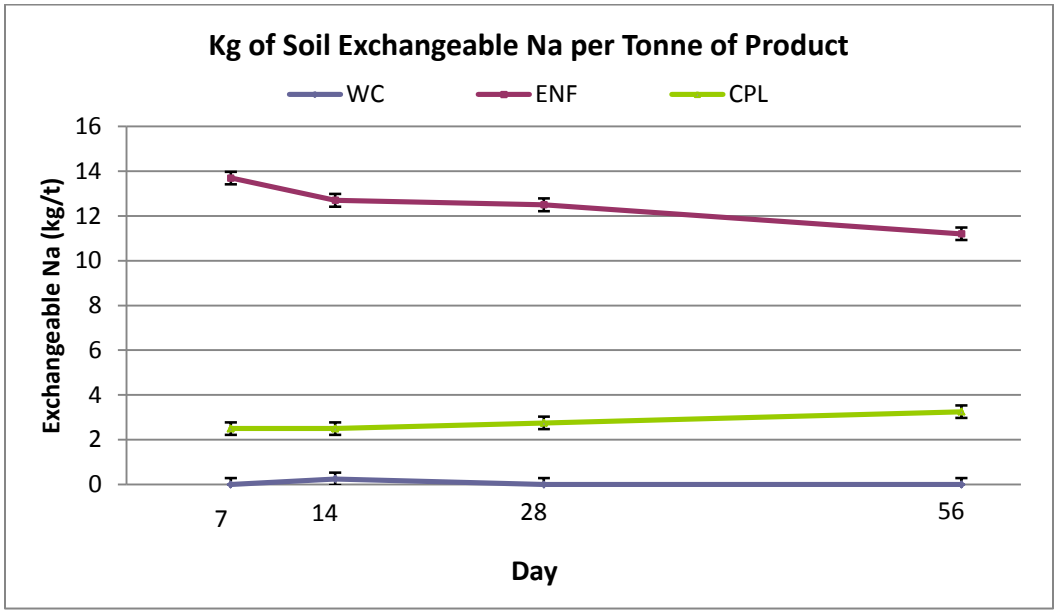

Figure 6: Soil exchangeable sodium per tonne of product applied over time. Error bars represent standard error.

By Day 14, soil pH decreased initially under both ENF and composted poultry litter, then increased by day 28 and subsequently decreased by day 56 (Figure 7). The worm castings treatment followed a similar trend. The differences in fluctuations among the fertilizers are probably related to soil nitrification, mineralization and/or salinity-related processes. The high pH of the control by day 56 is probably a reflection of lime inputs in the absence of fertilizers which acidify, such as the ENF and CPL.

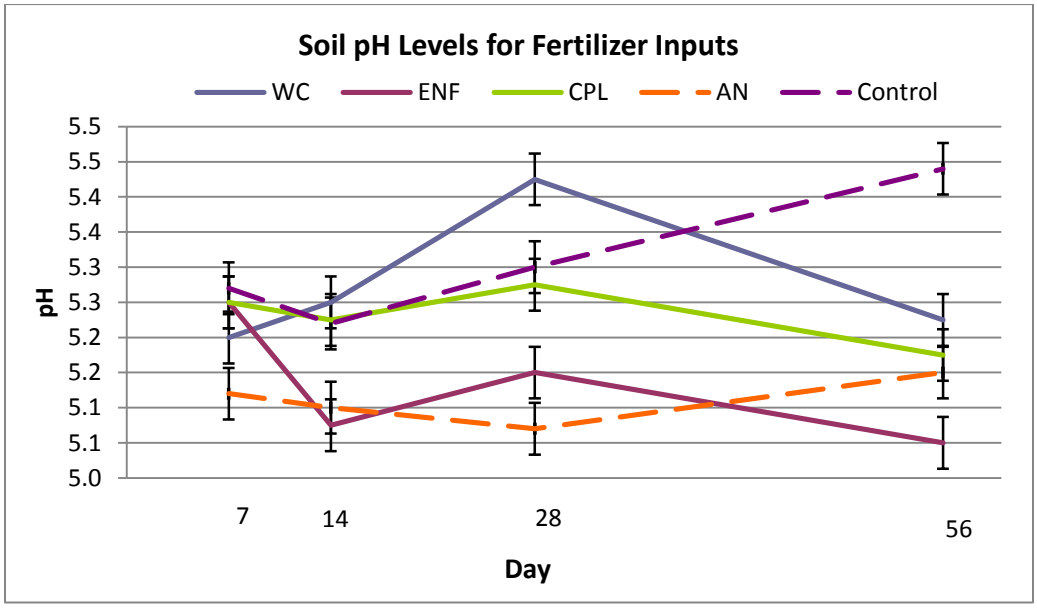

Figure 7: Soil pH levels resulting from the various fertilizers over time. Error bars represent standard error.

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Discussion

The ENF incubation results demonstrate high concentrations of N, P and K relative to other organic fertilizer sources (see Table 6). Analysis numbers summing to 10% is unusually rich for an organic fertilizer. ENF is comparable to composted poultry litter (CPL) in its N, P and K content, however, a higher proportion of the N and P in the ENF was available. Worm castings (WC) had very low levels of available N, P and K. The rapid mineralization and early availability of nutrients of ENF are indicative of its favorable C:N ratio. It is possible that with repeated applications of ENF, accumulated salts could be problematic in soils already high in salts. In the field study reported below, EC levels remained within the normally acceptable range for most crops.

Table 6: NPK Analysis of Enterra Natural Fertilizer. Certified analysis provided by SGS Agrifood Laboratories, Guelph, ON.

	Fertilizer NPK Analysis (%) DW Basis		
Fertilizer Type	Total N	Available P_2O_5	Soluble K_2O
ENF	4.66	2.40	3.00
CPL	4.19	1.80	2.90
WC	2.81	0.05	0.05
	% Available of Total*		
ENF	34	85	100
CPL	30	43	100
WC	4	16	17

*% Available N calculated based on day 56 incubation trial results

Field Trial

The field trial was designed to assess the effect of ENF on a wide variety of crop types (lettuce, leafy; Bok Choi, cruciferous; bean, leguminous; potato, tuber) and the impact on soil fertility under field conditions. Lettuce was included because of its potential susceptibility to wireworm attack.

The site at Amara Farm was selected due to its relatively low N, P, and K levels which should allow for observation of crop nutritional and soil fertility effects (see Table 4). The site, converted from pasture to cultivation for this study, was known to have wireworms, however at the time disease pressure was not deemed sufficient to compromise the nutritional study.

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Results

Upon removal of the jute sacks covering the soil – two weeks after ENF application and soil incorporation - in preparation for planting, a dense network of fungal growth was observed on the ENF-treated plots but not the controls (Picture 1 and Picture 2). This fungus has been identified as *Mucor circinelloides f. griseocyanus* and *Mucor circinelloides* f. *circinelloides* (ID provided by National Fungal Identification Service, Agriculture and Agri-Food Canada). Members of the genus *Mucor* are ubiquitous and found in soil, on plant surfaces and decaying vegetable matter, and in the digestive tracts of many animals.

Picture 1: Fungal networks were present only in the plots receiving ENF.

Picture 2: Close-up of fungal network.

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1st Planting Results

Soil Analysis – 1st Planting

Total Available Nitrogen (TAN = NH_4-N plus NO_3-N), exchangeable K, Na and salinity (EC) all showed significant and proportional responses to 0, 5 and 10 t/ha ENF applications (Table 7).

For available Bray P_1 only the plots designated for Bok Choi showed significant differences with increasing ENF application rate. Plots designated for the other crops failed to show this trend for unknown reasons. Correspondingly, Bok Choi was the only crop to demonstrate significantly increased tissue P concentrations with increasing ENF (see Table 8; Plant Tissue Analysis section below).

The amount of TAN provided by ENF applications closely matched the amount observed in the incubation trial (approximately 12-14 kg/t; see Figure 2). This validated the predictive value of the incubation study. Residual TAN maintained its positive response to ENF application. Values remained below the high risk level suggested to avoid nitrogen pollution (http://www.agf.gov.bc.ca/resmgmt/EnviroFarmPlanning/FV_SoilNutrientStudy/_FVSNS-CombinedReport_Feb28_2007_for_Release.pdf).

At the 10 t/ha rate, EC levels were still within the range generally regarded as safe for crop production (< 2 dS/m). By harvest, salinity levels had dropped substantially for all crops. This was probably related to crop uptake, and possibly leaching associated with heavy rain events during the growing period. (see relevant sections below).

Table 7: 1st Planting Field Crop Soil Fertility Results with Respect to ENF Applications. Numbers in bold are statistically significant at the 5% significance level.

1st Planting Field Crop Soil Fertility Results with Respect to ENF Applications

Field Crop	ENF Applications (t/ha)	At Planting Available (kg/ha) TAN	Bray P	Exch. K	Exch. Na	Residual TAN (kg/ha)	At Planting Saturated Paste pH	EC (dS/m)	At Harvest Saturated Paste pH	EC (dS/m)
Bean	0	**108**	61	**136**	**108**	**55**	5.3	**1.03**	5.8	**0.76**
	5	**177**	65	**230**	**145**	**68**	5.3	**1.33**	5.7	**0.97**
	10	**267**	68	**332**	**232**	**93**	5.3	**1.78**	5.7	**1.08**
	SE	9	2	18	17	8	0.1	0.11	0.1	0.06
Potato	0	**110**	58	**163**	**87**	**63**	5.5	**0.85**	5.2	**0.72**
	5	**158**	60	**253**	**154**	**78**	5.4	**1.53**	5.2	**0.91**
	10	**221**	69	**280**	**185**	**161**	5.5	**1.83**	5.1	**1.15**
	SE	3	5	14	8	8	0.1	0.20	0.1	0.10
Lettuce	0	**135**	46	**136**	**108**	**62**	5.2	**1.02**	5.3	**0.50**
	5	**209**	60	**230**	**146**	**75**	5.3	**1.40**	5.3	**0.54**
	10	**280**	57	**332**	**232**	**105**	5.3	**1.50**	5.1	**0.87**
	SE	12	4	18	17	7	0.1	0.12	0.1	0.06
Bok Choi	0	**143**	**51**	**136**	**85**	**48**	5.2	**0.98**	5.3	0.47
	5	**201**	**58**	**203**	**143**	**55**	5.3	**1.16**	5.4	0.50
	10	**263**	**68**	**294**	**201**	**92**	5.3	**1.61**	5.2	0.70
	SE	16	4	14	8	7	0.1	0.07	0.1	0.07

Plant Tissue Analysis – 1st Planting

Table 8 shows results of diagnostic tissue analyses. Despite, vigorous growth and healthy appearance that supported good yields, P and K deficiencies were recorded from diagnostic tissue analyses for lettuce and beans. No nitrogen deficiencies were detected in any of the tissue samples. Potato tissue

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nitrogen levels showed a statistically significant increase with increasing ENF application rate. Lettuce showed excessive N concentrations in the 5 and 10 t/ha treatments.

Lettuce and bean tissue analysis showed P deficiencies in all treatments on this P-deficient soil, and, along with potatoes, showed no P response to ENF applications. This corresponds to P availability in the soil analyses, which also failed to show increased P levels with increasing fertilization. P may be immobilized in this low pH soil rich in Al and Fe which tend to form insoluble complexes with phosphorus. However, in the bok choi plots where ENF raised soil P levels, this increase was reflected in the tissue analyses.

On this low K soil, all crops exhibited a positive K response to ENF applications: lettuce was only deficient in the control treatment, while beans had K deficiencies in the control and 5 t/ha treatments with adequate levels achieved in the 10 t/ha treatment. Bok choi had excessive consumption K levels associated with ENF applications of 5 and 10 t/ha.

Lettuce exhibited Ca deficiency despite ENF application. This was probably induced by the large amounts of K added to the soil by the ENF in the absence of adequate inherent soil Ca and Mg levels. The soil Ca:Mg and K:Mg ratios were greater than 8 and 2, respectively (data not shown). To alleviate these Ca and Mg imbalances, these soils would benefit from the use of dolomite lime or Sul-Po-Mag fertilizers.

Table 8: Diagnostic tissue analysis results. Diagnostic values taken from http://novascotia.ca/agri/documents/lab-services/analytical-lab-vegecroptissue.pdf. Values in bold are statistically significant at to 5% significance level.

Deficient Excessive

Field Crop	ENF Applications (kg/ha)		Total %				
			N	P	K	Ca	Mg
Bean	0		3.87	0.26	**1.09**	1.66	0.32
	5		4.07	0.24	**1.76**	1.79	0.27
	10		3.98	0.26	**2.02**	1.67	0.27
		SE	0.11	0.01	0.08	0.07	0.02
Potato	0		**3.96**	0.27	**2.02**	2.12	0.66
	5		**4.12**	0.29	**3.07**	2.57	0.69
	10		**4.44**	0.29	**3.20**	2.33	0.61
		SE	0.11	0.01	0.25	0.16	0.07
Lettuce	0		3.26	0.22	**1.92**	1.35	**0.52**
	5		3.49	0.17	**3.82**	1.20	**0.37**
	10		3.73	0.20	**4.33**	1.18	**0.37**
		SE	0.13	0.01	0.25	0.08	0.04
Bok Choi	0		4.15	**0.32**	**2.95**	4.06	**0.37**
	5		4.50	**0.50**	**4.95**	3.79	**0.28**
	10		4.62	**0.47**	**5.64**	3.51	**0.26**
		SE	0.13	0.04	0.30	0.21	0.01

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Growth, Development, and Yields – 1st Planting

First planting marketable yields of lettuce and bok choi were greatly affected by ENF application, with virtually no yield from the control plots (Figure 8). This effect was probably due to high mortality caused by wireworm (Figure 9, Picture 3) in the control plots; ENF may have provided wireworm protection to lettuce and bok choi plants in the first planting. Given that there was no statistically significant difference in the low mortality rates between the 5 and 10 t /ha bok choi plots, the statistically significant yield difference observed can be attributed to fertilization. For lettuce, there were no differences between 5 and 10 t/ha treatments either in mortality or yield, however both had higher than expected mortality of about 30%.

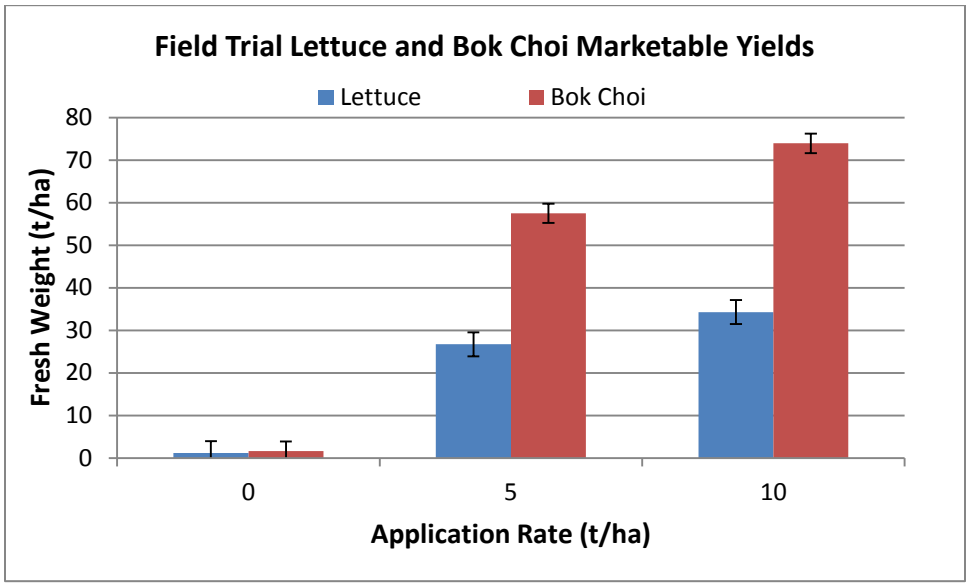

Figure 8: Marketable yields of bok choi and lettuce from the first planting. Error bars represent standard error.

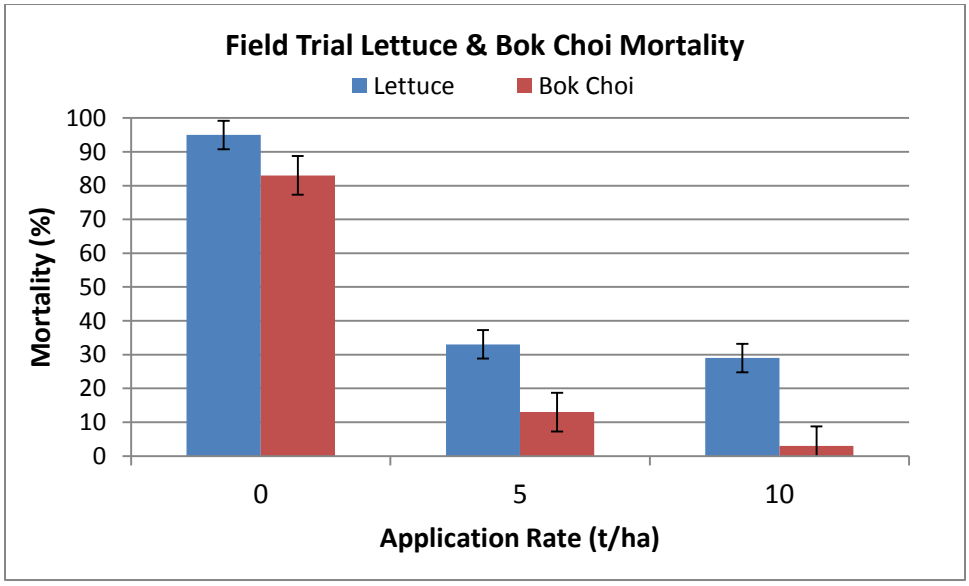

Figure 9: Mortality of lettuce and bok choi for the first planting.

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Picture 4: Lettuce mortality was near 100% in plots not receiving ENF. Green plants are in the plots where the ENF/frass was applied at either 5 or 10 t/ha, whereas the red wilted plants are in plots which received no ENF and have died.

There were no statistically significant ENF treatment effects on wireworm counts (Table 9), and lettuce mortality did not correlate with the number of wireworms present (data not shown); the possible wireworm inhibition effect of ENF may not be associated with a lower wireworm population. The observed number of dead wireworms was also very low. Note: the wireworm population present in the field trial soil was far above levels considered critical for economic damage (> 4 wireworms per trap). Such high wireworm populations are not uncommon in fields recently converted from pasture to cultivated crops, as was this field.

Table 9: Average wireworm trap counts in the field trial site. Both samples were taken at least 2 weeks after ENF was incorporated into the soil.

	0 t/ha	**5 t/ha**	**10 t/ha**
Lettuce (w/ plants)	25	15	19
Beans (no plants)	12	18	17

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A

B

C

D

Picture 5: Nearly all bok choi plants in controls in planting 1 were dead (A), whereas those in ENF treated plots were partially protected (B). Even healthy bok choi plants (those growing in ENF treated plots; C) often had wireworms feeding at their bases (D).

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Marketable yield of beans tended to decrease with increasing ENF applications while leaf and stem weights increased (Figure 10). The heavier vegetative growth observed in the 5 and 10 t/ha treatments were probably associated with delayed senescence induced by higher soil nitrogen levels. In addition, the late planting of the beans necessitated an early harvest due to weather conditions. There were no differences in mortality amongst the treatments, although wireworm damage to the cotyledons was present in all plots. ENF applicationwas not associated with lowered mortality or higher yield.

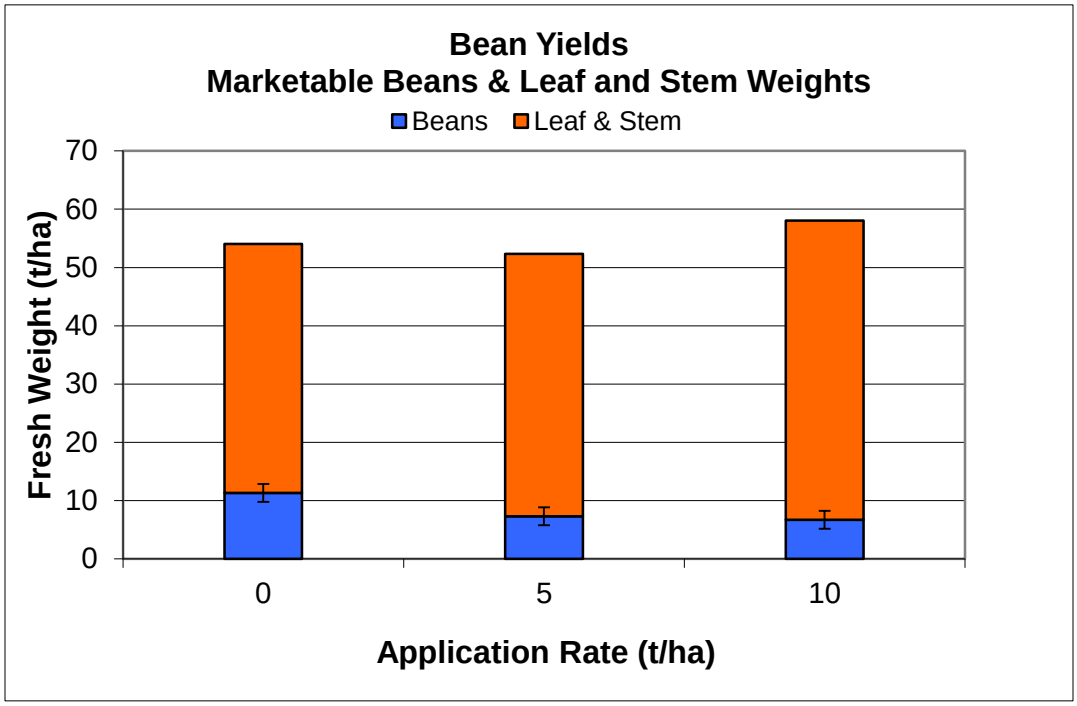

Figure 10: Bean yields across fertilizer treatment levels. Error bars represent standard error.

There was a statistically significant difference in potato tuber yield (weight) between the control and both the 5 and 10 t/ha treatment, but no further yield increase was obtained by applying 10 t/ha (Figure 11). The same results were obtained for above ground biomass (data not shown). There did not appear to be mortality differences among treatments; however, wireworm damage to tubers reduced marketable yield to zero across treatments. ENF was not associated with reduced tuber damage.

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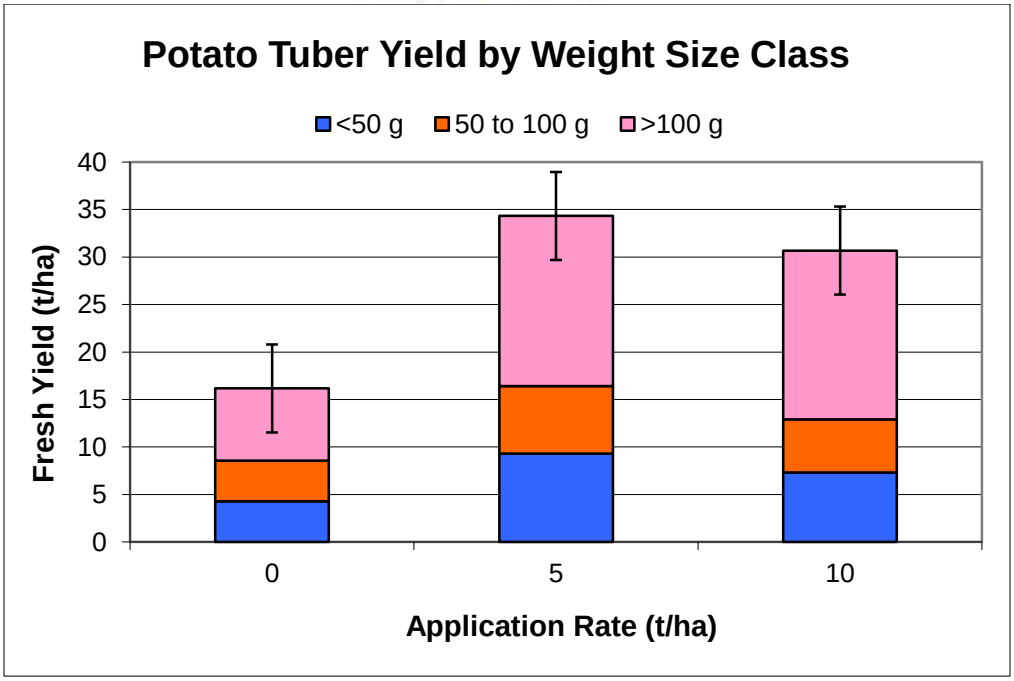

Figure 11: Potato tuber yields partitioned into size classes across fertilizer treatment levels. Error bars represent the standard error of the total tuber yields for each treatment.

Nutrient Budgeting/Uptakes –Bok Choi 1st Planting

Additional analyses to allow description of a nutrient budget were undertaken for the bok choi crop. Total available nitrogen (TAN) at planting for the 5 and 10 t/ha ENF applications were 200 and 250 t/ha, respectively (Figure 12). Crop removal (crop N uptake) plus residual soil N at harvest approximately equaled the amount available at planting. The values for the control treatments lacked significant N uptake due to high mortality. The low levels of residual TAN in the controls may be related to a number of factors but is most likely the result of leaching.

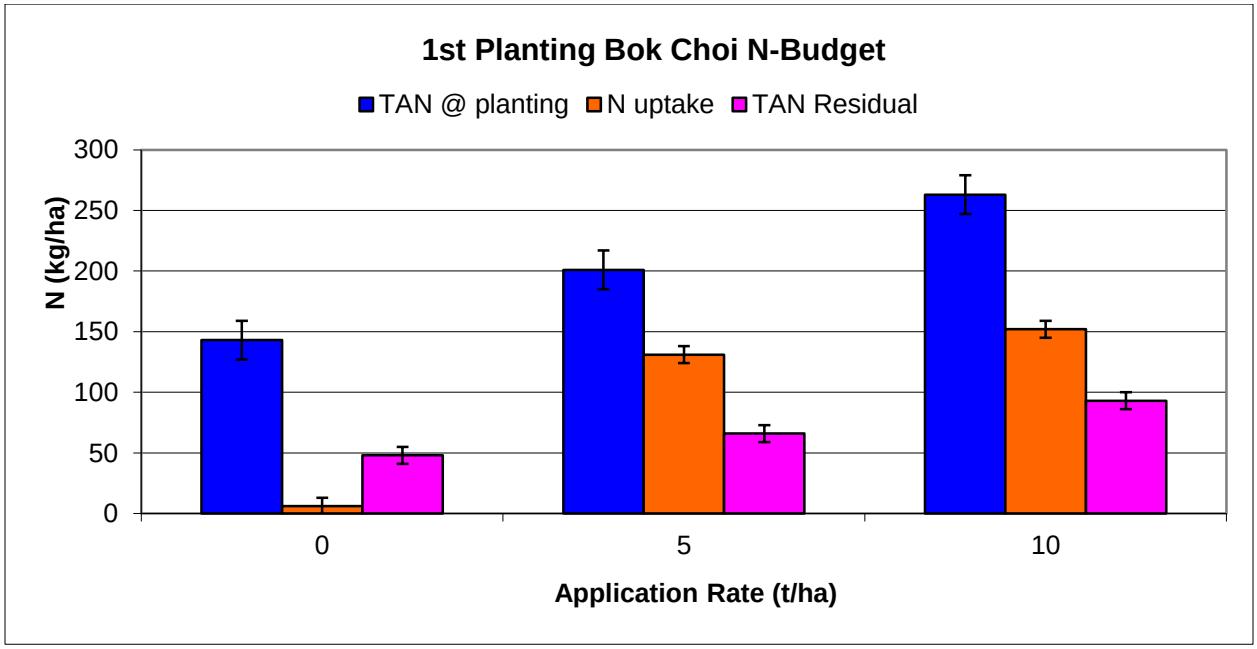

Figure 12: Bok Choi N budget. Error bars represent standard error.

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Bok choi NPK uptakes closely followed crop yields (Figure 13; Figure 8). The negligible uptake observed by the controls was due to poor plant survival at the site. There was little difference in NPK uptakes between the 5 and 10 t/ha applications of ENF. For both 5 and 10 t/ha applications, the NPK uptakes were approximately 140, 20, and 130 kg/ha, respectively. This indicates that a 5 t/ha application of ENF is probably sufficient for commercial yields of bok choi.

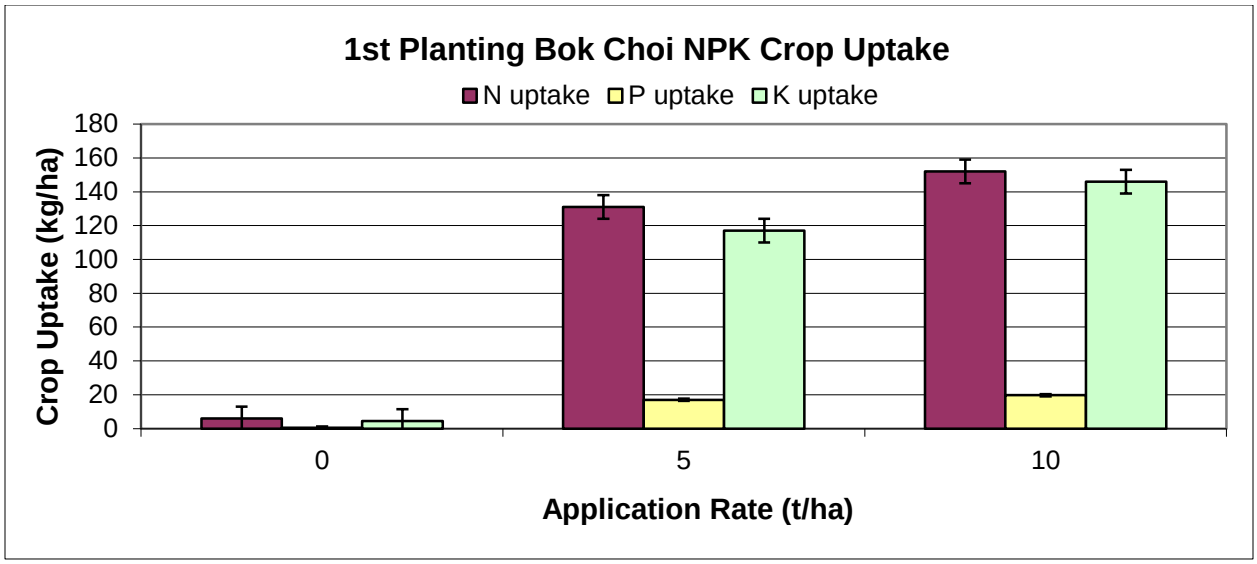

Figure 13: 1st Planting Bok Choi NPK Crop Uptake values. Error bars represent standard error.

2nd Planting Results

Upon removal of sacks one week after the second application of ENF, the fungus depicted in picture 1 and 2 (see above), was again evident only in the plots which had received the second application of ENF.

Soil Analysis – 2nd Planting

With reapplication of ENF at 5 t/ha, significant increases in TAN and exchangeable K were observed and these increases were proportional to the original 0, 5 and 10 t/ha applications of ENF (Figure 14). As observed for the first application, no such trends were evident for Bray P_1.

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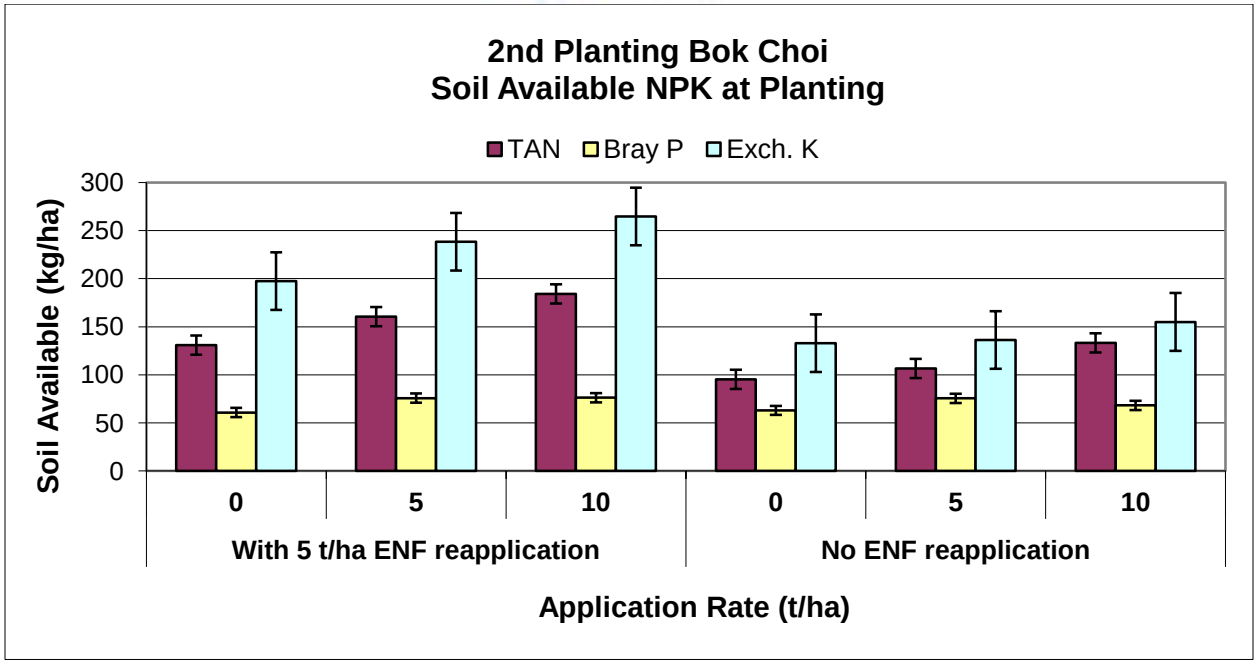

Figure 14: 2nd Planting Bok Choi Soil Available NPK at Planting. Error bars represent standard error.

Growth, Development, and Yields – 2nd Planting

Unlike the first planting, bok choi control plants demonstrated higher survivorship than lettuce, with high survival rates despite the wireworm infestation present at the site (Figure 15). Wireworms were observed feeding at the base of most bok choi plants, but they did not appear to cause mortality. Other unknown factors were at play that minimized the impact of wireworms on this Bok choi crop. Lettuce, on the other hand, demonstrated more acute mortality, even in ENF-treated plots, than was observed with the first crop planting. Initially, the reapplication of ENF did reduce mortality in the lettuce, but the effect was short lived. Mortality for lettuce with and without ENF reapplication was approximately 80 and 100%, respectively by 17 September. These contrasting results in the second planting when compared to the first planting are unexplained. In terms of differing methods, the field soil incubation period of the ENF prior to planting was two weeks for the 1st planting and one week for the 2nd planting.

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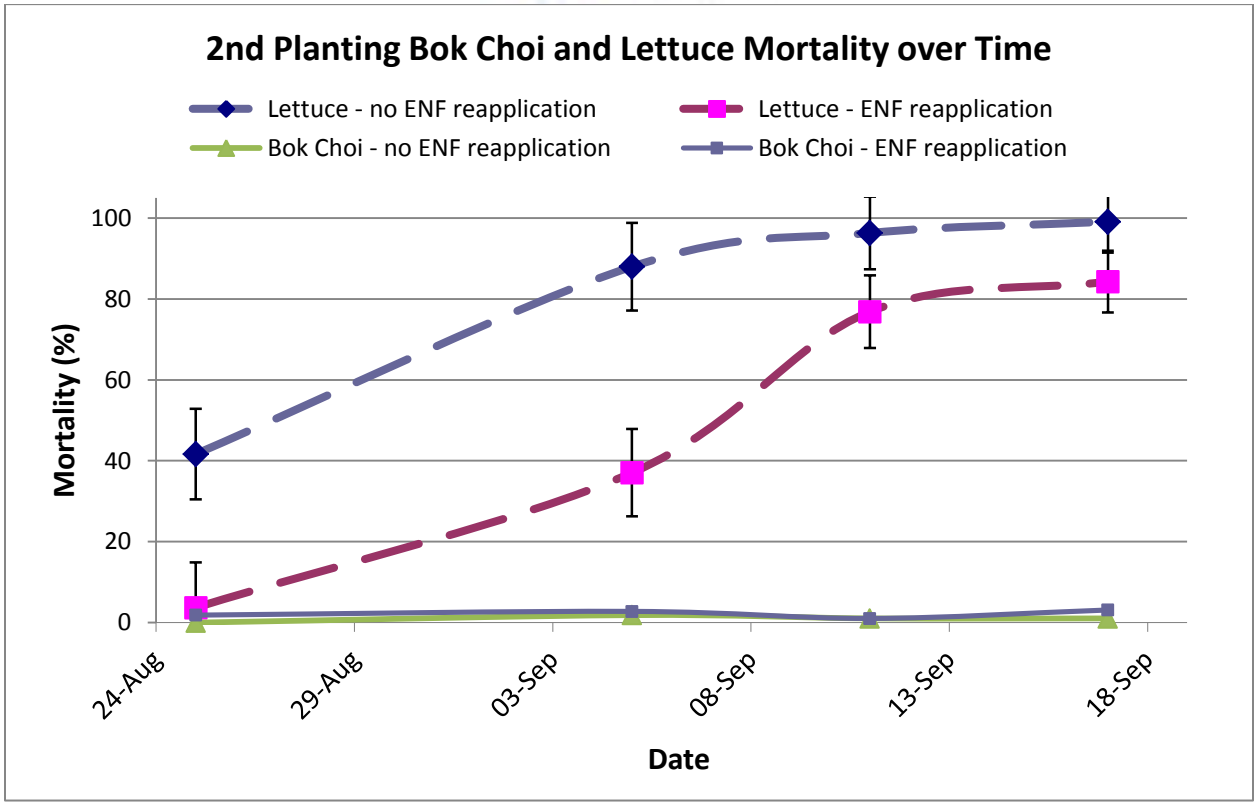

Figure 15: Mortality of lettuce and bok choi for the second planting. Error bars represent standard error.

Bok choi yields were significantly higher in plots receiving a second application of ENF at 5 t/ha (Figure 16). This application replaced nutrients taken up by the first crop or lost to leaching.

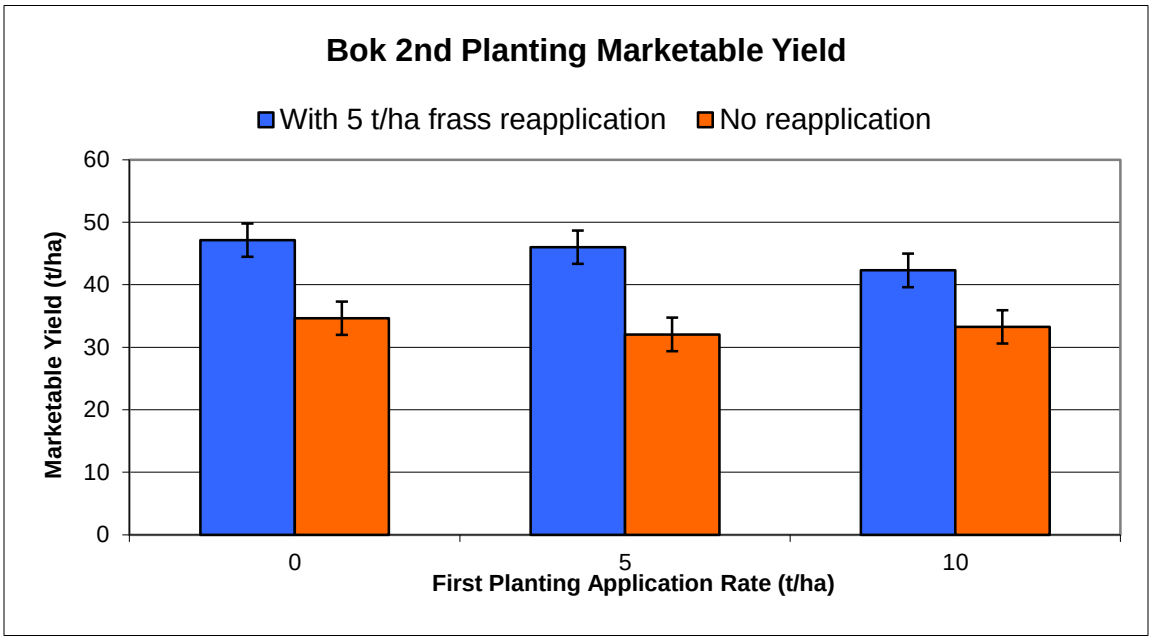

Figure 16: Marketable yields of bok choi from the second planting. Error bars represent standard error.

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Nutrient Budgeting/Uptakes – 2nd Planting

Bok choi NPK uptakes were reflective of both the soil nutrient and crop yield responses discussed above (Figure 14, Figure 16). N and K uptakes were significantly higher in plots receiving a second application of ENF at 5 t/ha. Significant NPK uptake response to the initial 0, 5, and 10 t/ha ENF applications were also restricted to the plots receiving a second 5 t/ha application. Plots receiving a second application of ENF had N uptakes ranging between 80 and 90 kg/ha, whereas plots without a second ENF application had N uptakes of only approximately 60 kg/ha. For K, plots receiving a second application of ENF took up between 60 and 90 kg/ha, whereas plots not receiving a second application took up approximately 40 kg/ha. As with soil available P, P uptakes appear to be unaffected by a second application of ENF across treatments.

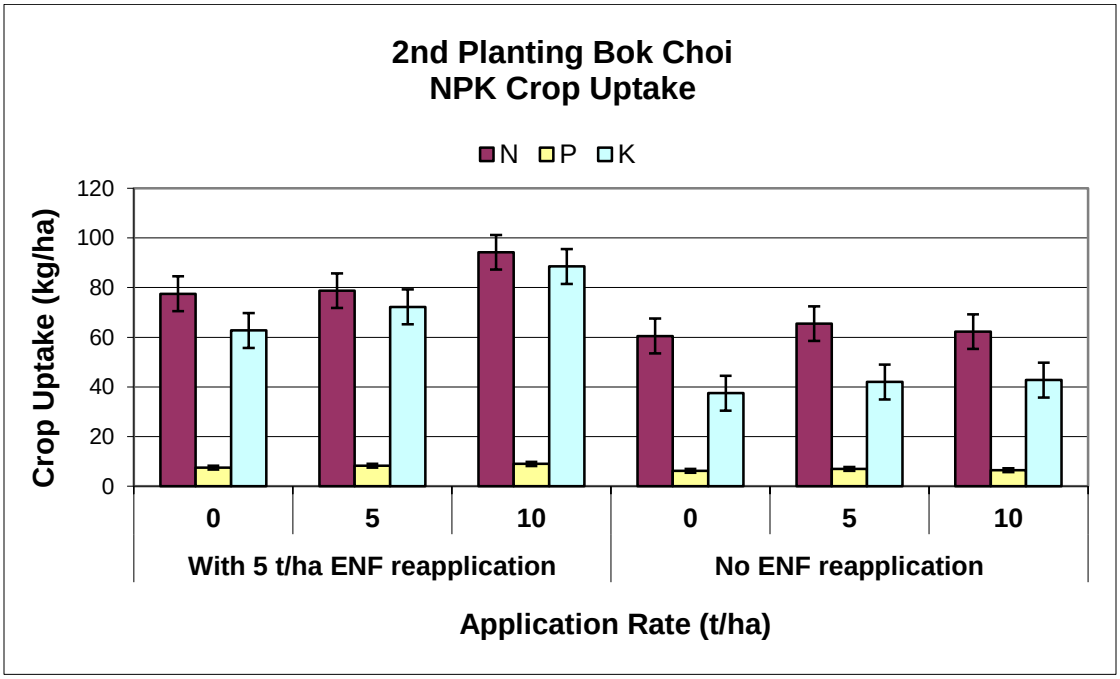

Figure 17: 2nd Planting Bok Choi NPK Crop Uptake values. Error bars represent standard error.

Discussion

Soil nutrient concentrations in the field trials are consistent with the availability predicted by the incubation study. Nitrogen levels are significantly elevated in the treated plots, with NH_4-N representing the largest increase. Soil analyses next spring would be useful to assess the residual levels of available nitrogen and phosphorus from these applications.

The marketable yield of the bok choi and lettuce plantings indicates a significant increase in yield in the treated plots compared to the control plots which experienced high mortality. Fertilizer effects were thus confounded, however differences between 5 and 10 t/ha application rates, with similar mortality, may be attributable to nutritional differences.

In 2 of the 6 plantings in this field experiment, ENF was associated with reduced crop damage. The high mortality in control plots was associated with wireworms, although conflicting wireworm trap counts show no evidence of reduced wireworm populations in treated plots. The mechanism through which ENF appeared to decrease mortality in first planting lettuce and bok choi is unknown. Increased vigour

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of the crops may have allowed them to withstand wireworm feeding until well established. Alternatively, ENF may have somehow reduced wireworm activity. The trap and core samples taken from the plots provided little evidence of dead wireworms. ENF may have acted as a deterrent. Uniformly damaged potato tubers and bean cotyledons across all treatments suggest that the wireworms were neither repelled nor deterred. The *Mucor* fungus that became so evident shortly after ENF application or some other fungus associated with ENF applications may be having some effect. Insect frass has long been claimed to exhibit pest control properties, anecdotally and in product advertising. The claimed effects are usually ascribed to the presumed chitin content of shed insect integument that remains in the insect digestate (frass). However, the biological chitin, chitosan and/or chitinases activity and its potential effects as a biological control agent for ENF is unknown. To the authors' knowledge, there have been no known variations, discoveries and/or documented results that have definatively demonstrated the practical or potential use of BSF frass/soil fertilizer as a biological control to wireworm in pest management. The potential biological control of wireworm using ENF observed at Amara Farm was subsequently disclosed to Todd Kabaluk (wireworm specialist; Pacific Agri-Food Research Center, Agassiz, BC); he is conducting further research into the potential biological control mechanisms of wireworm. A protective effect of ENF product could be an important development for an industry currently lacking any effective means to counter one of our region's most prevalent pests.

Starter Trial

The starter trial was intended to test the effect of ENF as a component of an organic seedling growing medium. The organic industry lacks a diversity of approved products available for growing media and in the absence of information many growers formulate their own mixes. An organic product capable of meeting the nutritional needs of seedlings in such a medium would be of value to many producers. Worm castings are a common component of organic soil media and were chosen as a comparison in this study. A wide range of application rates was selected to provide a response curve.

Results

Seedlings showed a positive yield response to increasing applications of ENF (Figure 18). Yield responses to ENF were much greater than those observed with similar rates of worm castings. Applications of ENF in excess of 15 t/ha (for bok choi, onions, beans, and tomatoes) or 10 t/ha (for lettuce and squash) resulted in decreased above ground biomass.

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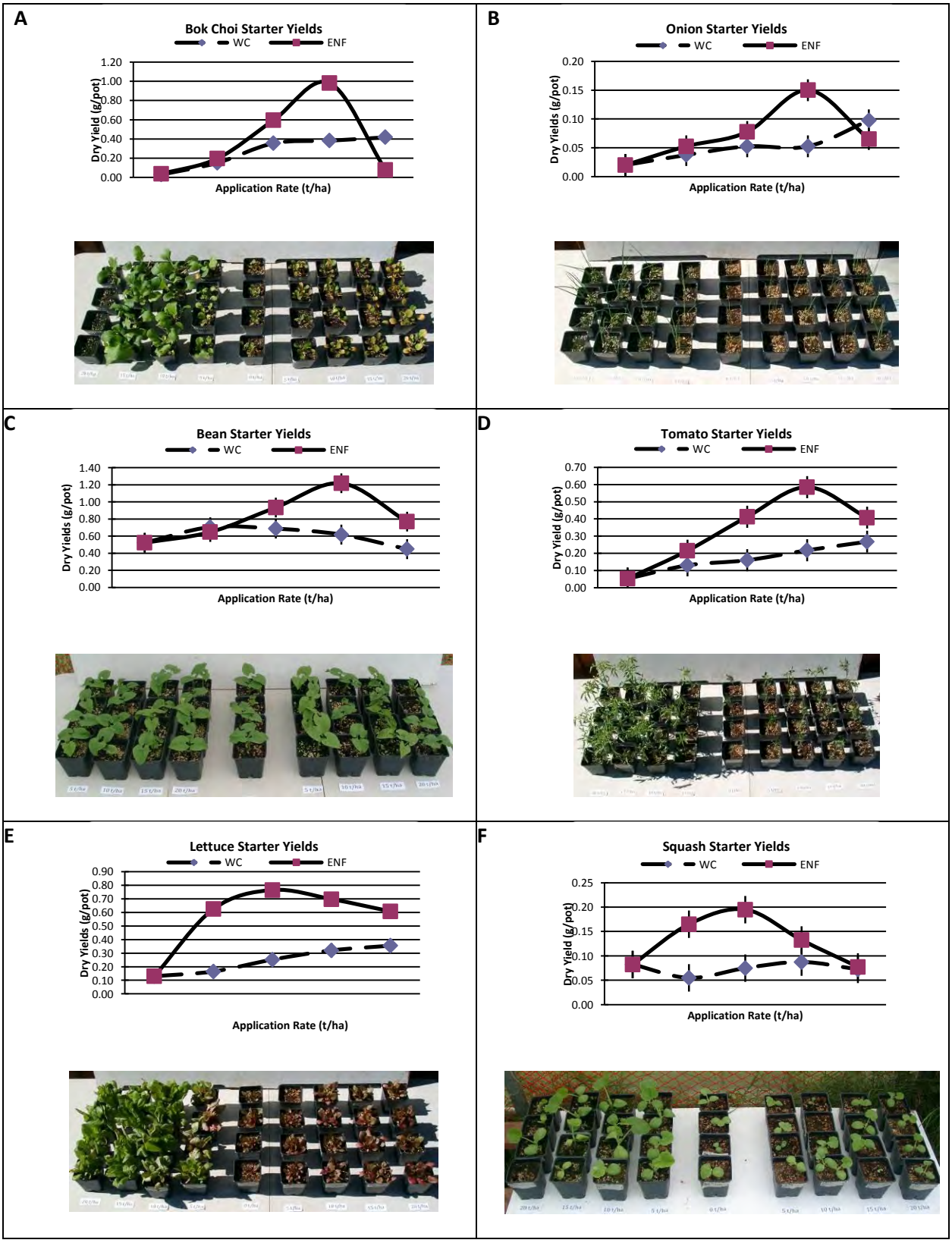
Figure 18: Starter trial biomass yields and related photos. In photos, ENF treated starts are on left, worm castings on right; control is center column. Error bars represent standard error.



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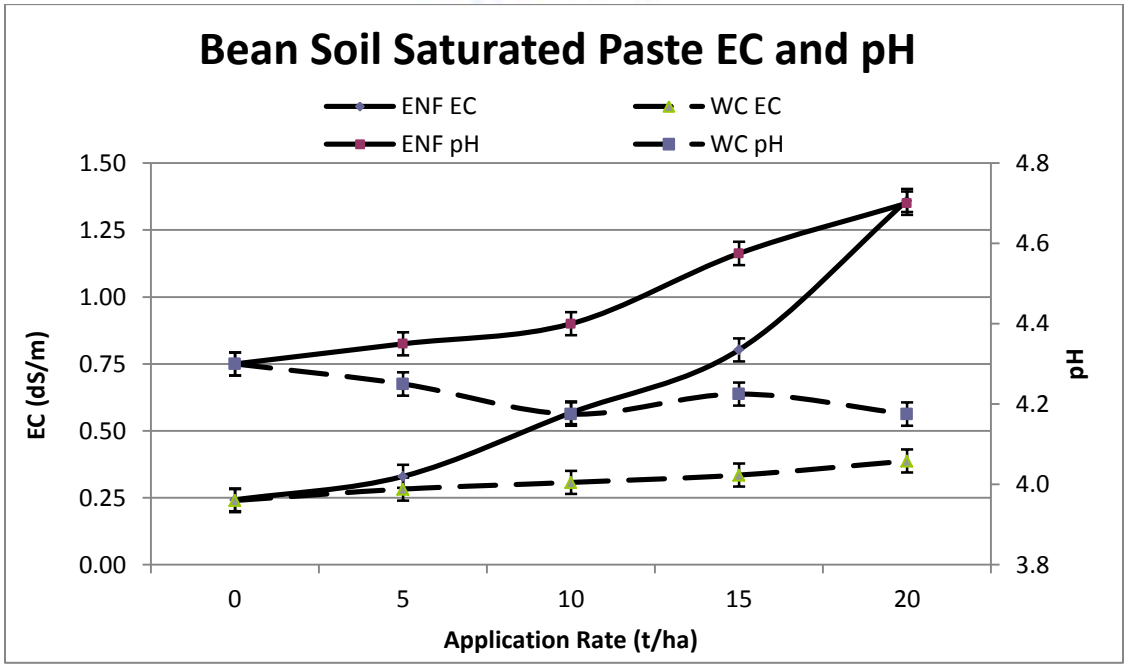

Figure 19: pH and salinity of the bean starter potting medium under ENF and worm castings across fertilizer application rates. Error bars represent standard error.

The pH and salinity of the ENF-treated bean starter potting medium increased markedly with increasing applications of ENF, while no major differences were observed in the worm castings-treated medium. The increased pH probably reflects increasing levels of NH_4-N mineralization processes while increased salinity is probably related to higher levels of sodium at higher application levels. The EC levels observed at the high end of ENF application in this trial were below known toxicity levels; the reasons for plant toxicity associated with ENF applications of 15 - 20 t/ha (Figure 18) are unclear at this time,but may relate to NH4-N toxicity.

Discussion

Inclusion of Enterra Natural Fertilizer at 5 to 8% DW/DW (equivalent to 10 to 15 t/ha) in peat/perlite starter plant media supports plant growth superior to that of worm castings applied at similar rates. Many producers currently use worm castings as a component of their starter mixes. Relative to worm castings, the most beneficial results achieved by ENF at lower application exemplified ENF as a useful soil or potting growth media amendment.

Conclusions and Recommendations

ENF is a well balanced fertilizer comparable to composted poultry litter, but with higher available N and exchangeable K and a narrow C:N ratio that promotes rapid mineralization when applied to soil. In the relatively low fertility soil at the test site, field application rates of 5 t/ha supported commercial yields of bok choi, lettuce and potatoes. Some evidence in this investigation suggests that ENF may, under some circumstances, provide some wireworm protection. However these results were not confirmed by a second ENF application. More research is needed to determine possible mechanisms. Inclusion of ENF at 5 to 8% DW/DW in peat/perlite starter plant media supports plant growth superior to that of worm castings applied at similar rates. More trials are required to determine optimum use and application method, rate and timing for specific crops and soil conditions.

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