EFFECTS OF BIODYNAMIC PREPARATIONS ON SOIL, WINEGRAPE, AND COMPOST QUALITY ON A CALIFORNIA VINEYARD

By

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A thesis submitted in partial fulfillment of

the requirements for the degree of

MASTER OF SCIENCE

WASHINGTON STATE UNIVERSITY
Department of Crop and Soil Sciences

DECEMBER 2003

To the Faculty of Washington State University:

The members of the Committee appointed to examine the thesis

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ACKNOWLEDGMENT

This research was supported by Fetzer Winery, Jim Fetzer, Benziger Family Winery, the Land Institute, the Biodynamic Farming and Gardening Association of North America, and the Dept of Crop and Soil Sciences at Washington State University. I would like to thank my advisor, John Reganold, for his uncanny ability to locate funds right at the opportune moments and for his humor and patient editing and support throughout. Special thanks go to Lynne Carpenter-Boggs who in many ways stood in as my co - advisor and without whose help I could not have completed this work. I would also like to thank Alan York as major consultant to vineyard operations and his expertise on all matters pertaining to biodynamic and ecological viticulture and Will Brinton for advice on the design of the composting experiment and help with data interpretation. Also, thanks go to Glen McGourty for providing the yield analyses. Many thanks go to my committee members Dave Bezdicek and Dave Granatstein for approving and supporting this research. Special thanks go to Chad Boardman for on site management of the composting experiment and to Mary Fauci, Debbi Bikfasy, Margaret Davies, and Eric Harwood for much needed advice and assistance in the laboratory. Final thanks go to Ron Bolton for his invaluable help with endless computer related questions and problems.

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Abstract

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December 2003

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Biodynamic preparations were investigated for their effects in soil quality, winegrape quality and the quality of grape pomace and manure compost in a seven year study (1996 to 2003) on a California vineyard. The biodynamic field sprays (500, 501 and barrel compost) were not shown to have any effect on soil quality. Analyses of the winegrapes showed the biodynamic treatment to have significantly higher tannins in 2002 and Brix in 2003. In addition, biodynamic winegrapes contained notably higher tannins, total phenols and total anthocyanins in 2003. When earthworms were presented with biodynamically sprayed or unsprayed soil, significantly more worms migrated to the biodynamically sprayed soil.

Grape pomace and manure compost treated with biodynamic preparations 502 through 507 showed increased ammonium concentration, higher dehydrogenase activity and increased growth response of wheat seedlings in 2002. Compost in 2003 developed a high pH, lost substantial N, and supported less aerobic microbial activity. The biodynamic preparations were not shown to have an effect during these adverse composting conditions.

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BIODYNAMIC AND ORGANICALLY MANAGED VINEYARDS

BIODYNAMIC COMPOSTING OF WINEGRAPE POMACE

General Introduction

Biodynamics was first presented as an ecological approach to agriculture in 1924 by the Austrian philosopher Rudolf Steiner (Steiner 1993). While embracing many of the traditional approaches of organic production, such as crop rotations and soil building regimes using compost, biodynamic farmers see their farms as resembling an actual living organism or individual entity (Koepf 1989, Koepf 1990, Sattler and Wistinghausen 1992, Beismann 1997, Vereijken et al. 1997). Biodynamic farms are managed as a diversified system of animal and crop production interspersed with natural habitat breaks (Koepf 1989, Koepf 1990, Beismann 1997, Vereijken et al. 1997). In addition, the farm is operated as close to a self-sufficient system as possible with all fertilizers and animal feeds produced on the farm (Pfeiffer 1983, Koepf 1990, Sattler and Wistinghausen 1992). To achieve this, a farm is designed so that the ratio of pasture animals and cropland reflects the actual carrying capacity or load limit of the land in that particular region, so as not to deplete the soil (Pfeiffer 1983). Self-sufficiency in balance with the entire ecosystem is attempted, as opposed to simply maximizing yields (Koepf 1989).

In order to promote efficient cycling of nutrients, a series of eight fermented herbal and manure based preparations numbered 500 through 507 (Koepf 1989) are applied regularly, in small quantities to the soil, compost and crops. The claim is that these preparations help to stimulate the vitality of the soil and plant, hence improving the health of the animals and the quality of the crops produced (Pfeiffer 1983). These substances are not claimed to act as fertilizers, rather to harmonize naturally occurring environmental processes enabling the plant to grow in a balanced way (Koepf 1989). In addition, some biodynamic farmers plant their crops according to specific phases of the

moon, maintaining as the moon causes tides in the oceans, sap flow in a plant is also influenced by the moon and planets (Koepf 1989).

Although there are some detractors, (Kirchman 1994) biodynamic farming, with its strong emphasis on soil building holds many benefits in terms of sustainability and soil quality. (García et al 1989, Foissner 1992, Reganold et al. 1993, Droogers and Bouma 1996, Granstedt and Kjellenberg 1997, Carpenter-Boggs 2000). Whether the preparations themselves have any additional benefits is more controversial, however. Research involving use of the preparations indicates that there may be benefits to soil quality and crop quality (Koepf 1993, Reganold 1995) although results are mixed. This research will be discussed in greater detail in the following chapters.

The increase in organic and biodynamic farming practices is also spreading to the wine industry with 1.5 % US grape acreage now certified organic (Green 2003). Around 500 ha of winegrapes are certified biodynamic in the U.S. (H. G. Courtney, Josephine Porter Institute, 2003, personal communication). Biodynamic wines have received growing attention in the past ten years as some of the world's most prestigious appellations have adopted the method (Macle 1998, Blackburn 1999, Mathews 2000, McInerney 2002, Brown 2003). Growth in biodynamic viticulture has been particularly fast in France, with 1000 hectares of winegrapes cultivated biodynamically in 1993 and 15,000 hectares in 1998 (Meunier 2001).

Winegrapes could be the ideal crop in which to study differences in fruit quality resulting from management practices, because there is already a considerable body of knowledge available on the subject due to the high profile nature of wine quality. A

surprisingly small amount of work, however, has addressed the issue of whether organic let alone biodynamic farming practices influence winegrape quality.

A study in the Mosul valley grape-growing region of Germany showed differences between 50 and 300 % in biological soil quality between biodynamic and conventional vineyards. Biodynamic vineyards contained higher organic matter, dehydrogenase activity, microbial biomass and earthworm populations (Gehlen et al. 1988). Organic matter additions have been shown to reduce the incidence of a wide array of plant pathogens (Lazarovits 2001). A comparison study between organic and conventional vineyards (Lotter et al. 1999) showed that increasing organic matter in the soil significantly reduced effects of Phylloxera infestations. Even though Phylloxera populations were similar under both systems, fungal necrosis as a result of the damage was 70 % lower under organic management.

Lowered yield, frequently associated with organic practices, has been linked to better quality winegrapes (Jackson and Lombard 1993). This is because more resources are allocated to the reduced crop load by the vine resulting in increased sugar and flavor of the grapes. Soil that is overly fertile can also affect quality as vines respond by producing a vigorous canopy that shades the grapes and may even cause microclimates conducive to disease (Jackson and Lombard 1993). High levels of nitrogen and potassium have also been shown to adversely affect grape composition and wine quality (Jackson and Lombard 1993). A long-term study comparing organic and conventionally grown grapes found few consistent differences in grape quality (Henick-Kling 1995). The differences detected appeared to relate to the crop load of the vine: Organic vines brought

a smaller crop to higher sugar and better color content in adverse years. In good growing seasons though there were no significant differences.

This study measured the effects of the biodynamic preparations on soil quality, winegrape quality and the quality of winegrape pomace and manure compost. The study took place on a commercial Demeter certified biodynamic vineyard near Ukiah, California. Four replicated blocks of merlot grapes were established and two treatments applied, biodynamic preparations and no biodynamic preparations as the control. Apart from the use of the preparations all management practices were the same. Soil quality was monitored over a period of six years and crop quality was measured in the final four years. The compost experiment was carried out over two years with six replicates.

This thesis is divided into four chapters. This general introductory chapter describes the background and objectives of the research. Chapter 2 presents data on soil quality for the first six years of the study and data on winegrape analyses for the last four years. Chapter 2 is to be submitted to the *American Journal of Enology and Viticulture* and is formatted according to the requirements of that journal. Chapter 3 presents the results from two years of composting of grape pomace with and without the biodynamic preparations. This chapter will be submitted to *Compost Science and Utilization* and is formatted accordingly. A final conclusion chapter summarizes the results found in chapters 2 and 3 and suggests areas of future research. Jennifer Reeve is solo or first author on all chapters; Lynne Carpenter-Boggs, John Reganold, Alan York, William Brinton and Glen McGourty are co-authors and are listed according to their contributions to the research.

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Soil and Winegrape Quality

in Biodynamic and Organically Managed Vineyards

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Biodynamics, a unique form of ecological agriculture, involves a whole system approach of integrating the farm as a self regulating organism with a diversity of crops, animals, and wildlife areas. In addition a series of fermented preparations are used, said to stimulate the soil and enhance plant health and quality of produce. Although the benefits of such integrated systems are no longer denied, the question of whether the preparations themselves have any effect on soil and crop quality is controversial. Biodynamic wines have received considerable international acclaim in recent years with many of the world's best known wineries adopting the practice. Whether high quality winegrapes are the result of organic management as a whole or

whether an additional effect can be attributed to the preparations is unknown. For this reason, a long-term, replicated, 4.9-ha study was instigated in 1996 on a commercial vineyard to investigate whether any effects on soil and winegrape quality could be detected as a result of the use of the biodynamic preparations. The study consisted of eight plots in a randomized, complete block design with two treatments, biodynamic and an organic control. All management practices were the same in all blocks, except for the addition of the preparations to the biodynamic treatment. No differences were found in soil quality in the first six years. However, in a separate earthworm behavior experiment, where earthworms were given the choice of migrating to biodynamically treated or nontreated soil, significantly more worms migrated to the biodynamically sprayed soil. Analyses of the winegrapes showed the biodynamic treatment to have significantly higher tannins in 2002 and Brix in 2003. In addition, biodynamic winegrapes contained notably higher tannins, total phenols and total anthocyanins in 2003.

Key words: biodynamic viticulture, organic viticulture, soil quality, winegrape quality.

Biodynamic agriculture was developed in the1920s in response to concerns from farmers about the deteriorating soils and health of their farms (Steiner 1993). A form of ecological agriculture, biodynamics involves considerably more than eliminating synthetic chemical fertilizers and pesticides. A systems-based approach, biodynamics holds a unique view of each farm as an organism. This includes a strong emphasis on

soil building and diversity of crops, animals and wildlife habitat (Koepf et al. 1990). In addition biodynamic practitioners use a series of fermented manure, plant and mineral-based preparations (Table 1), said to stimulate nutrient cycling and promote photosynthesis and optimal compost development.

Although biodynamic farming has been shown to be a viable and productive farming system (Reganold et al. 1993, Reganold 1995, Mäder et al. 2002), the question as to whether the preparations themselves have any contributing effects on soil or crop quality is another matter. Most studies on this topic have been conducted by biodynamic proponents, have not been published in refereed journals, compare biodynamic with conventional practices only and have variable results (Koepf et al. 1993, Reganold 1995). Nevertheless, a number of studies have overcome some of these shortfalls.

A 21-year study comparing biodynamic, organic, integrated, and conventional systems showed not only the highest biological activity in the biodynamic plots but suggested that the biodynamic system might be more efficient, as indicated by higher microbial diversity correlated to lower soil respiration (Mäder et al. 2000, 2002). The biodynamic and organic treatments were not identical, however, as fertilizer was applied to the biodynamic system in the form of compost and to the organic system in the form of half rotted manure (Mäder et al. 2000).

Results from a second long-term study (Raupp 2001) showed that over time soil organic carbon decreased under the conventional treatment and to a lesser extent the organic treatment; only the biodynamically treated plots retained their original levels of organic carbon. A four-year study conducted in Germany found significantly higher organic matter, total N, dehydrogenase activity, microbial biomass, and dehydrogenase

per unit microbial biomass in the biodynamic treatment compared to both the organic and conventional treatments (Abel 1987, as translated by Koepf 1993). Similarly, Goldstein (1986) demonstrated that biodynamic preparations positively influenced biological soil properties and crop root growth. Colmenares and Miguel (1999) found that the preparations, sprayed on permanent grassland in Spain over 3.5 years, increased dry matter content in the absence of any fertilization. Bourguignon and Gabucci (2000) studied paired plots of winegrapes in France, either treated or untreated with the biodynamic preparations and, although they found no differences in the surface soil, there was greater microbial activity in the subsoil of the biodynamically treated plots. In a double-blind study involving biodynamic, organic and conventional wheat and potatoes, biodynamic wheat showed best baking quality (Deffune et al. 1990). Neuhoff et al. (1998) found slightly higher yields of potatoes in the biodynamic as opposed to organic plots in three consecutive years.

In addition some studies have focused on the effects of individual or groups of preparations. Carpenter-Boggs et al. (2000c) found that biodynamic preparations caused significant effects on compost development. The silica preparation 501 was shown to induce systemic resistance against pathogens in cucumber (Schneider and Ullrich 1990). Fritz and Köpke (2000) found that horn silica 501 significantly increased growth of green beans alone and in combination with plant extracts. König (2000) showed that oak bark preparation 506 significantly lowered mildew infection in zucchini. Goldstein and Koepf (1982) found that the preparations influenced the nutrient status of wheat seedlings grown in nutrient solutions. Deffune and Scofield (1994) showed that biodynamic preparations stimulated the growth of wheat seedlings at a concentration as low as 10^{-25}. Conversely,

a study by Carpenter-Boggs et al. (2000a and 2000b) comparing biodynamic with control plots was inconclusive as were studies in Sweden (Pettersson et al. 1992) and Australia (Penfold et al. 1995).

Biodynamic wines in particular have received considerable international attention in recent years with many top wine producers converting to biodynamics (Macle 1998, Blackburn 1999, Mathews 2000, McInerney 2002, Brown 2003). Interest in France has been particularly strong with more than 110 well-known domaines convinced biodynamics is the best approach in viticulture and winemaking (Meunier 2001). Growth has been fast with 1000 hectares of winegrapes cultivated biodynamically in France in 1993 to 15,000 hectares in 1998 (Meunier 2001). In the USA interest is also growing, with nearly 500 hectares of vineyards certified biodynamic, such as Benzinger, Bonterra, Ceago and Frey, and with more experimenting with the method (Brown 2003, H. G. Courtney, Josephine Porter Institute, 2003, personal communication). Vineyards, such as Benzinger, Bonterra, Ceago and Frey, are all practicing biodynamic methods.

Master wine taster and author Jancis Robinson said that "successful biodynamic wines do taste different – wilder, more intense and dangerous" (McInerney 2002). And Mike Benziger of Benziger Family Vineyards noted that the percentage of wine produced with potential for his estate blend increased from 52 to 100% in three years after conversion to biodynamics (Walker 2003).

Since no research has currently been published in a refereed journal on the biodynamic method when applied to viticulture, we began a long-term replicated field experiment in 1996 comparing biodynamic and organic (the control) winegrape production on a commercial vineyard in Mendocino County, CA. The objective was to

determine whether any changes in soil and winegrape quality could be detected as a result of using the biodynamic preparations.

Materials and Methods

Experimental Site and Management. The experimental area was 4.9 ha, part of a 60-ha biodynamic vineyard (*Vitis vinifera,* L. cv. Merlot, grafted onto 5C rootstock) at McNab Ranch, near Ukiah, CA. The commercial vineyard, owned by Bonterra, was organic from 1994 to 1996 and started its transition into biodynamics in 1996. Since 1999, the vineyard has been both Demeter (biodynamic) certified and organically certified by U.S. National Standards.

Four 0.6-ha replicate plots for each of the two soil management systems were delineated in June 1996 in a randomized complete block design in the study area. Each plot contained about 50 rows (on average about 27 vines per row), with vines being trained to a vertical shoot position. The vines were planted in 1994 at a spacing of 1.8 m within rows and 2.4 m between rows, resulting in an average of 2233 vines per ha. The 114 cm of average annual precipitation at the site are supplemented with an under-vine drip irrigation system and a solid-set overhead sprinkler system for frost protection and heat cooling. Vines were irrigated regularly in years up to 2000 but from 2001 continuous irrigation was discontinued. In addition, since 2001 expected yields were reduced to 9 Mg per ha.

The soil in the study area is a Cole loam, drained (fine, mixed, thermic Pachic Argixeroll), that formed in alluvium. In 1996 (prior to implementation of management treatments) soil profiles were examined in several places in each of the eight plots for

morphological characteristics. The soil morphological characteristics examined were depth and thickness of soil horizons based on texture, gravel content, structure, and color. No differences between plots within each block were found. Soil samples were also taken at 0 to15, 15 to 30, and 30 to 45cm from each of the designated plots in 1996. Each sample consisted of a composite of 10 subsamples, 5 taken from between the vines and 5 from the center of the rows. All subsamples were taken randomly from the inner thirty rows of each experimental plot and 7.6 m from row ends to minimize edge effects. Analyses of pertinent physical, chemical and biological properties revealed no significant differences between treatments at that time (data in appendix). This lack of differences in soil morphological, physical, chemical, and biological properties is essential at the start of such a field experiment (Reganold, 1988).

The two treatments received identical soil and vine management practices throughout the experiment, except for the biodynamic preparations, which were only applied to the biodynamic plots. Biodynamic plots annually received biodynamic spray preparation 500 in spring and fall, preparation 501 twice in summer, and barrel compost spray in the fall (Table 1). A cover crop of spring rye, a high biomass crop, was sown in both treatments and disked under to increase organic matter in the soil in 1996. In 1997, grape pomace and manure compost with or without the biodynamic preparations was applied to all plots at a rate of 3 to 4 kg per hectare. Alternating rows of clover and wildflowers to attract beneficial insects and an oat, mustard and clover green manure crop served as cover crops in both treatments.

Soil Analyses. Soil samples were taken at 0 to15 cm in fall 1997, spring 2000 and fall 2001 and 2002. Samples were shipped to Woods End Research Laboratory (WERL,

Mt. Vernon, ME) from California by overnight mail. The samples were passed through a 2 mm sieve and stored at 4ºC until analyses. Soil pH was measured in a 1:2 w/v in water and $CaCl_2$ (Eckert and Simms 1995). Samples were checked for the presence of free carbonates by reaction with hydrochloric acid. Organic matter was determined using the Walkley Black method (Shulte 1995). Biological CO_2 respiration was measured over a 1 wk incubation at 34ºC (Anderson 1982) and expressed as g kg^{-1} C and total CO_2 output. Solvita Soil Life was measured in 2001 and 2002 (W. F. Brinton, WERL, 2003). Potential nitrogen release was estimated based on 1.5 % mineralization of total N released in top 0 to 15 cm of soil (W. F. Brinton, WERL, 2003, personal communication). Water stable aggregates were measured as the volume of soil still aggregated after wet sieving (Kemper and Rosenau 1986). Texture was determined using the hydrometer method (Gee and Bauder 1989). Conductivity was measured using the saturated paste method (Gartley 1995). Nitrate was measured using ion chromatography (Griffin et al. 1995). Available and reserve P was extracted with Bray P 1 and 2 and measured by atomic absorption spectrometry (Wolf and Beele 1995). Exchangeable calcium, Mg and Na was measured using atomic absorption spectrometry and extracted with the modified morgan extractant (Wolf and Beele 1995). Soluble chloride and sulphate were measured by ion chromatography. (Singh et al. 1995). Exchangeable Mn, Zn and Cu were measured using atomic absorption spectrometry and the modified morgan extractant and boron was measured using the hot water method (Simms 1995). Cation exchange capacity was calculated from the measured cations according to Ross (1995).

In the fall of 2002 the following additional characteristics were analyzed at Washington State University (WSU): Ammonium - N and NO^-_3 – N were measured in a filtered extract of 5 g soil in 25 ml 1M KCl on a Latchett QuickCHem FIA+ 8000 series autoanalyzer using the salycilate method for NH_4 and the NH_4CL_2 method for NO_3. Readily mineralizable carbon (RMC), basal respiration (BR) and active microbial biomass by substrate-induced respiration (SIR) were measured according to Anderson and Domsch (1978). Ten g of wet weight soil was brought to 23.6 % moisture content and incubated at 24ºC for 10 days. Total CO_2 released after 10 days was considered RMC. Vials were recapped for 2 h and the hourly rate measured for BR. For SIR 0.5 mL of 30 g L^{-1} aqueous solution of glucose was added to the same soil samples, rested for 1 h before being recapped for 2 hrs. Carbon dioxide was measured in the headspace using a Shimadzu GC model GC –17A, with a thermal conductivity detector and a 6', 1 / 16'' HaySep 100 / 120 column. Dehydrogenase enzyme activity was measured using 2.5 g dry weight soil and phosphatase enzyme activity using 1 g dry weight soil as described by Tabatabai (1994). All enzyme reactions were measured using a Bio-Tek microplate reader model EL311s. Potential nitrification and aerobic N mineralization were measured as described by Schmidt and Belser (1994) using 10 g moist weight soil. Nitrate - N and NH_4^+ - N after 10 days incubation were measured on a Latchett QuickCHem FIA+ 8000 series autoanalyzer using the NH_4CL_2 and salicylate methods.

Humus polymerization was estimated using E4/E6 and E2/E3 ratios, electron transition absorbance bands (Δ_{ET}) and extinction coefficients. For these tests 4.5 g of soil was extracted in 45ml of deionized water, 0.5 M NaOH or 0.1 M $Na_4P_2O_7$ under N_2 for 24 h. The resulting extracts were filtered, neutralized to pH 8 with HCl and absorbance

measured at 665, 529, 465, 373, 365, 350, 280, 272 and 250 on a Perkin Elmer Lambda 2 UV/VIS spectrometer. The E4/E6 ratios were calculated by dividing the 465 by the 665 nm wavelengths. The E2/E3 ratios were obtained from the 250 and 365 nm wavelengths. Electronic transition absorbance bands were calculated using the equation $\Delta_{ET} = 2.18(\text{In}(A_{280} / A_{350}))^{-1/2}$ (Pokorná et al. 2001). Extinction coefficients were calculated using the equation: (529 nm x extraction vol.) / dry weight soil (Richter and Wistinghausen 1981). Laboratory measurements were carried out in triplicate with the exception of the humic acid extractions, which were run in duplicate.

Earthworm populations were measured in Spring 2003 by removing three 20 cm^3 samples from each plot and then counting the number of earthworms. A worm behavior experiment was carried out. A fine-silty mixed, mesic Pachic Ultic Haploxerol surface soil (0 to 20 cm) was collected. Sixteen hours before the experiment half the soil was treated by lightly spraying with 2 g L^{-1} of the biodynamic preparation 500 and 3 g L^{-1} barrel compost that had been stirred for 1 h. The rest of the soil was sprayed with an equal amount of water. Four 50 by 30 cm cardboard boxes were prepared having two treatment compartments separated by a 10 cm neutral zone. One section of each box was filled with 3.5 kg biodynamically treated soil, the other end with control soil. An additional 10 ml of preparation 500 and barrel compost solutions were added to the biodynamically treated soil and water to the control soil in each box. Forty earthworms on average from the vineyard in California were placed in the soil free neutral zone where they could migrate to either section through notches in the dividers. The number of worms migrating to the biodynamic treatment was recorded after 24 h and converted to a

percentage of the total number of worms placed in the neutral zone. The experiment was repeated and the data combined together with an initial single box trial.

Vine Nutrition and Winegrape Analyses. Fifty mature leaves with petioles were selected, sixth or seventh from the growing tip from healthy vines in the middle of each block. Samples were taken at veraison in the second week of September 2003, and analyzed for total N, P, K, Ca, Mg, Br, Zn, Fe, Cu and Mn (WSALPT 1997). Grapes were sampled from ten contiguous vines in two rows for a total of twenty samples from the middle of each block. Samples were sent to Enologix (Sonoma, CA) in 2000, 2001, 2002 and 2003, for analysis in style (eg Bordeaux), Q5-Index, ratio or aging potential, total phenols, tannins, monomers, free anthocyanins, total anthocyanins and complex anthocyanins. The Q5-Index is calculated by comparing the average biochemical analysis of grapes to a database of analyses of top vintages. The exact calculations and compounds used in the analysis are proprietary to the Enologix company (Sonoma, CA. www.enologix.com).

Statistical Analyses. All statistical analyses were run using paired t-tests and Minitab Statistical Software Release 13.32. For the worm behavior experiment a simple one sample t-test ($n = 9$) was used to determine whether the percentage of worms preferring the biodynamic treatment differed from 50 %. All statistical differences represent $p < 0.05$ unless otherwise stated.

Results and Discussion

Soil Quality. No consistent significant differences were found between the biodynamically treated and untreated plots for any of the physical, chemical or biological

parameters tested (Tables 2, 3 and 4). Also, no differences were found in the biological quotients: dehydrogenase activity per unit CO_2 respiration (BR), dehydrogenase activity per unit readily mineralizable carbon (RMC), respiration per unit microbial biomass (SIR) or SIR per unit RMC (Table 4). Biological quotients are often a more sensitive measure of microbial efficiency than measurements made in isolation (Anderson 1994). Mäder et al. (2002) found significantly higher microbial efficiency in biodynamic compared to organic plots using this method. Carpenter-Boggs et al. (2000b) also investigated these quotients but found no differences between biodynamic or organic plots.

Results from analysis of extracted humic substances showed only one statistically significant difference ($p<0.05$) for electronic transition absorbance (Δ_{ET}) extracted in water, with the untreated soil having the higher value (Table 5). Higher Δ_{ET} and absorbance at 272 nm is associated with higher aromaticiy, conversely a lower E2/E3 ratio is associated with higher aromaticity (Pokornà et al. 2001). The E4/E6 ratio has been correlated to macromolecular size and to a lesser degree oxygen content with a lower ratio indicating a larger molecule with lower oxygen content (Chen et al. 1977). Kononova (1966) used E4/E6 ratios to differentiate between soils of different origins but found it less sensitive to differentiating between closely related soils. Richter and Wistinghausen (1981) could differentiate between biodynamically and conventionally managed soils using extinction coefficients.

There are a number of possible reasons why no differences were observed in the soils between treatments. Since soil in the plots on the experimental site was a fertile, alluvial Mollisol, compost was only applied once (in 1997) to the plots of the experiment to avoid

over vigor in the winegrapes from high soil fertility. The biodynamic method does in most farming circumstances require the regular application of compost. Biodynamic compost is treated with specially fermented plant-based inoculants (preparations 502-507) and there is evidence that the preparations alter the microbial community and end product of the compost as a result (Carpenter-Boggs et al. 2000c). Lack of regular compost applications may have reduced potential differences between the soil treatments.

In a review Raupp and König (1996) show that biodynamic preparations cause the greatest effect under poor yielding conditions, a small effect under medium yielding conditions, and no, or inhibiting effect under high yielding conditions. Yield conditions include available nutrients soil and climatic conditions. McNab Ranch has good soils and climate.

The observation that some soils respond in a given way while others may not is by no means an idea unique to biodynamics. Beyer et al. (1999) found that the typical and widely accepted observation that microbial carbon will rise and dehydrogenase activity decline in response to nitrogen application, was not the case in the three soils they tested. Bergstrom et al. (1998) found increased enzyme activity as a result of conversion to no-till practices in a fine-textured soil, but no such response in a coarse-textured soil.

Six years may be insufficient time for treatment differences to manifest in soil. It is usually claimed that effects of the preparations are slow to fully develop. It should also be noted that Bourguignon and Gabucci (2000) found no differences in topsoil but increased activity in the subsoil under biodynamic treatment relative to the control. Soil below 15 cm was not evaluated in this study.

Taken as a whole a number of soil parameters changed over time. Several parameters increased in 1997 as a result of the initial compost application and initial spring rye cover crop. Organic matter spiked in 1997 and then fell back to pre-compost levels by 2000 (Figure 1). Water-stable aggregates were significantly different in all years, indicating the highly variable nature of this parameter (Figure 1). Calcium, Mg, total bases and cation exchange capacity were all significantly higher in 1997 and then declined slowly through 2002 (Figures 2 and 3). Available P and Na increased also in 1997, peaking in 2000 and then declining again (Figure 2). Overall, no change in P or K was found, however, and levels remained adequate for healthy vine growth (Figure 2). Potential N release rose significantly from initial 1996 levels and was maintained at around 55 kg/ha (Figure 4). Conductivity was significantly higher in 1996 and lowest in 2000; no problems with salinity are indicated.

Although some researchers (Nguyen et al. 1995, Penfold et al. 1995) have expressed concern that organic forms of agriculture may not adequately replace nutrients such as P and K, we did not observe declines in these nutrients over the duration of this study. With the exception of K grapes are not a nutrient demanding crop and a single application of compost was sufficient to influence soil fertility for several years. Although trace elements were very low in the surface soil (Table 3), no deficiencies were seen in the vines. It should be noted that surface soil testing does not reflect the minerals available to such a deep-rooted plant as winegrapes, whose roots can grow below 3 m where conditions allow (Campbell and Fey 2003).

Biodynamic practitioners often claim that earthworms increase after a farm is converted to biodynamics (Pfeiffer 1983). Earthworms are known to enhance soil

structure, organic matter decomposition, and nutrient cycling (Edwards and Lofty 1977). Although biodynamic plots contained 28 % more earthworms than control plots (Table 4), but high variability made these results statistically similar. In an experiment in which earthworms were allowed to migrate either to soil sprayed with BD preparations or soil sprayed with water only, a significant difference in worm preference was found for the biodynamic treatment (percentage = 70.22 %, $p = 0.011$, raw data in appendix).

Pfeiffer (1983) in a similar study also found that significantly more worms migrated to biodyamically treated soil. In a second experiment more earthworms migrated to soil sprayed with the valerian preparation 507 compared to unsprayed control soil. More recent experiments have shown earthworms to display avoidance behavior from soils contaminated with sub-acute levels of toxicants (Yeardley et al. 1996, Haimi and Paavola 1998). Earthworms are highly sensitive to their environment and may be indicators of small positive or negative changes in soil quality.

Vine Nutrition and Winegrape Analyses. It is possible that a more active rhizosphere could facilitate uptake of nutrients by the plants. To test this theory a leaf and petiole tissue analysis was carried out in 2003. There were no differences found between the treatments. Nutrition was found to be adequate in all plots; most elements were within recommended ranges (Table 6). The exceptions were K, Cu and Mn, which were all low. However, samples were taken at veraison, when ripening fruit becomes a sink for nutrients, especially K. No deficiency symptoms were seen in the vines at any time. Total N was high confirming that there was no shortage of N.

Biodynamically treated grapes had significantly higher tannins ($p = 0.05$) in 2002 and in 2003 higher Brix sugars ($p = 0.03$), (Table 7). In 2003 a number of parameters were of

notable significance: total phenols ($p = 0.06$), tannins ($p = 0.06$) and total anthocyanins ($p = 0.06$). According to tannin and total phenol contents the style of grapes in both treatments was determined to be Style III, which is the highest quality Bordeaux style wine given by Enologix (http://www.enologix.com). Style III indicates that wine made from these grapes has the potential to be a long-lived wine that will improve with age. The Enologix Q5-Index is based on a scale of –1 to 1. Grapes from both treatments reached an Index of 0.95 in 2003, which is the best quality. The Q5-Index gives an indication of the flavor and color of the resulting wine based on chemical analyses of top vintages in previous years. The Ratio is also a measure of the longevity of the wine and again is based on correlations with analysis of top vintages. A score above 0.8 is correlated to wines of world class standard. In 2003 the grapes reached a ratio of 0.92 in both treatments.

Total phenols and tannins are part of the style rating. Tannin content above 2000 mg L^{-1} is considered undrinkable but should be above 1000 mg L-^{1} for top quality wines. Monomers are a measure of grape bitterness. Monomers over 1150 mg L^{-1} would produce bitter wines and values in excess of 1300 mg L^{-1} would be undrinkable; values below 500 mg L^{-1} are considered flabby, however, with no texture. Free and complex anthocyanins are important in color development and are also part of the style rating. Free anthocyanins, which age at 1000 mg L^{-1} per month, are less stable than complex anthocyanins, which only age at a few mg L^{-1} per month. Complex anthocyanins are usually present at 0 to 200 mg L^{-1}, the higher the concentration the better (http://www.enologix.com).

These data show a dramatic increase in grape quality, from average in 2000 to world class in 2003. Concurrently differences between biodynamic and unsprayed treatments have increased. These changes correlate with cessation of weekly irrigation and culling of yields starting in 2001. A reduction in quality in 2002 resulted from excessive temperature in the vineyard. Scientists have criticized Enologix for refusing to identify the exact compounds and methods used in their quality indexes (Neuman 2001). However, the Enologix method has repeatedly been shown to accurately predict wine critics evaluations and as a result is widely used by small wineries seeking an edge in a competitive market (Arrhenius et al. 1996, McCloskey et al. 1996, Neuman 2001).

One argument frequently cited against biodynamics is the unlikelihood that the small quantities of preparations used could affect plants or soils. However, many growth stimulators, regulators and hormones are known to be active at extremely low concentrations. For example the growth regulator triacontanol was shown to be active in promoting both shoot and root growth at concentrations of 2 to 10 ug L^{-1}, whereas the higher concentration of 20 ug L^{-1} had an inhibiting effect (Tantos et al. 1999). This effective concentration is similar to the usual application of preparations at 1 to 2 ug L^{-1} (Pfeiffer 1983). Notably, the field spray preparations 500 and 501 contain high levels of cytokinin like plant growth regulators (Stearn, 1976).

Recent research in quorum sensing has shown that bacteria regulate their population density by secreting tiny amounts of signaling substances. These molecules trigger gene responses in neighboring bacteria causing enhanced production of the signal molecule thereby magnifying the response through the population (Miller and Bassler 2001, Brelles-Marino and Bedmar 2001). When the signal reaches a critical threshold a host of

gene responses in the population are activated. Bacteria also use signal molecules to control competing bacteria of different species (Miller and Bassler 2001). Plants have been shown to produce bacterial signal mimicking compounds thereby regulating bacterial populations on their roots and leaves (Brelles-Marino and Bedmar 2001). If the BD preparations affect plant or microbial growth their activity may be due to such hormones and signal molecules.

Conclusions

No differences in soil quality at 0 to15 cm were found between biodynamically treated and untreated plots. Also, no differences were found in microbial efficiency as measured by biological quotients. It is possible due to the naturally fertile nature of the study site that conditions were not ideal for observing biodynamic effects in soil. In addition, insufficient time may have elapsed for any effects to manifest. In a separate earthworm behavior experiment, significantly more worms migrated to the biodynamically sprayed soil than to nontreated soil.

Compost was applied only once at the commencement of the study because of concerns of over-vigor of the vines. The effects of a single application of compost on soil parameters could be detected for several years. This suggests that only minimal compost application to fertile soils is needed to achieve lasting benefits in a low-intensity, high-quality cropping system such as winegrapes.

Leaf and petiole tissue analyses found adequate nutrition and no differences between treatments. Quality as determined by Enologix rose sharply as a result of culling grapes and ceasing regular irrigation. Grapes in 2003 received top quality ranking in

both treatments. Biodynamic grapes in 2002 had higher tannins and in 2003 higher Brix sugars, total phenols, tannins and total anthocyanins. This differences between the biodynamic and organic treatments coincide with the cessations of irrigation in the vineyard.

That no differences attributable to the biodynamic preparations were detected in the soil parameters measured in the vineyard study is consistent with previous work in that effects have been recorded in some situations but not in others. A number of effects linked to the use of preparations in previous studies were not addressed in this study, however, and would be of interest to evaluate. These include stimulation of root growth and the resulting associated changes in the subsoil. The possibility that the preparations act on particular species or groups of microbiota would also be of interest to evaluate. In addition, an evaluation of wine made from the two treatments is the next major step needed in determining whether the high quality observed in biodynamic wines is a result of use of the preparations, the vineyard management, or a combination of both.

Acknowledgements

This research was supported by Fetzer Winery, Jim Fetzer, Benziger Family Winery, The Land Institute, the Biodynamic Farming and Gardening Association of North America, and the Department of Crop and Soil Sciences at Washington State University. Special thanks go to Chad Boardman for managing the field site. Many thanks to Mary Fauci, Debbi Bikfasy, Margaret Davies and Eric Harwood for much needed advice and assistance in the laboratory. And final thanks to Ron Bolton for assistance with numerous computer related problems and questions.

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Table 1 Biodynamic preparations main ingredients and amounts used on 0.4 ha land or 13.6 Mg compost.

Preparation	Main Ingredient	Use	Unit volume (cm^3)	Unit Mass (g)
500	Cow (*Bos taurus*) manure	Field spray	35	38
501	Finely ground quartz silica	Field spray	2	1.8
502	Yarrow blossoms (*Achilliea millefoilium* L.)	Compost	15	1.1
503	Chamomile blossoms (*Matricaria recutita* L.)	Compost	15	3.0
504	Stinging nettle shoots (*Urtica dioica* L.)	Compost	15	4.4
505	Oak bark (*Quercus robur* L.)	Compost	15	3.9
506	Dandelion flowers (*Taraxacum officinale* L.)	Compost	15	4.7
507	Valerian flower extract (*Valeriana officinalis* L.	Compost	2	1.2
Barrel Compost	Cow manure fermented with 502-507	Field spray	47	51

Table 2 Means and standard errors (n = 4) of soil analyses (0-15 cm) conducted in 1997, 2000, 2001 and 2002.

	1997		2000		2001		2002	
	BD	Control	BD	Control	BD	Control	BD	Control
pH in H2O	7.1 ± 0.04	7.2 ± 0.08	7.2 ± 0.09	7.1 ± 0.06	6.7 ± 0.04	6.7 ± 0.02	7.0 ± 0.0	7.2 ± 0.05
pH in CaCl2	6.8 ± 0.07	6.8 ± 0.07	6.4 ± 0.03	6.3 ± 0.05	6.6 ± 0.13	6.7 ± 0.03	6.8 ± 0.08	7.6 ± 0.82
Organic Matter g kg^{-1}	38 ± 1.2	36 ± 2.3	24 ± 0.3	25 ± 1.0	22 ± 1.7	23 ± 1.7	23 ± 1.5	23 ± 1.5
Solvita Soil Life					3.5 ± 0.24	3.1 ± 0.14	3.5 ± 0.33	3.0 ± 0.47
Total CO_2 Output mg kg^{-1} wk^{-1}			377 ± 68	501 ± 26	1062 ± 182	812 ± 72	1125 ± 250	850 ± 268
Established Nitrogen Release kg ha^{-1}	0.0 ± 0.0	1.1 ± 0.58	50.1 ± 8.1	66.3 ± 3.1	62.9 ± 8.3	48.9 ± 4.3	64.4 ± 11.7	48.9 ± 13.2
Water Soluble Aggregates g kg^{-1}	540 ± 11	580 ± 40	46 ± 20	60 ± 17	450 ± 77	457 ± 43	95 ± 07	187 * ± 26
Gravel %	21.3 ± 6.0	22.0 ± 4.2	19.0 ± 2.9	24.0 ± 2.4	20.0 ± 4.1	23.5 ± 4.2	20.2 ± 1.7	22.2 ± 2.1
Sand %	41.7 ± 1.9	36.3 ± 1.2	51.7 ± 0.7	49.7 ± 1.9	47.0 ± 1.4	50.7 * ± 1.3	46.0 ± 2.4	50.7 ± 1.7
Clay %	12.0 ± 1.0	18.3 ± 2.6	20.0 ± 1.6	23.75 ± 0.3	18.7 ± 0.7	17.5 ± 2.0	25.0 ± 3.9	24.0 ± 5.3
Silt %	24.7 ± 3.4	23.3 ± 1.8	28.2 ± 1.7	26.5 ± 1.9	34.5 ± 1.8	31.5 ± 0.6	29.5 ± 2.9	25.0 ± 3.6
Conductivity mmhos cm^{-1}	0.2 ± 0.00	0.2 ± 0.00	0.09 ± 0.00	0.09 ± 0.01	0.15 ± 0.03	0.15 ± 0.02	0.18 * ± 0.01	0.17 ± 0.01
Available Phosphorus mg kg^{-1}	22.3 ± 7.3	17.3 ± 2.6	37.5 ± 3.4	31.5 ± 2.0	17.5 ± 0.3	17.7 ± 1.0	12.5 ± 0.6	13.2 ± 1.3
Nitrate mg kg^{-1}	11.7 ± 0.3	11.0 ± 1.5	1.4 ± 0.4	1.7 ± 0.3	15.0 ± 2.1	14.0 ± 1.0	6.0 ± 0.5	6.7 ± 0.7

Reserve Phosphorus mg kg^{-1}	34.7 ± 1.3	36.0 ± 8.7	57.7 ± 8.0	64.2 ± 6.6				
Chloride mg kg^{-1}	14.7 ± 1.3	14.7 ± 1.9	2.7 ± 0.3	4.0 ± 1.2				
Sulphate mg kg^{-1}	10.0 ± 2.0	9.0 ± 1.0						
Potassium mg kg^{-1}	202 ± 10	270 * ± 22	197 ± 9	177 ± 8	251 ± 73	198 ± 17	227 ± 9	245 ± 24
Sodium mg kg^{-1}	46 ± 2	72 * ± 7	144 ± 77	55 ±2 7	32 ± 1	33 ± 1	24 ± 2	22 ± 0
Calcium mg kg^{-1}	2356 ± 189	3867 * ± 369	2407 ± 61	2375 ± 64	2213 ± 29	2273 ± 113	2034 ± 110	1993 ± 52
Magnesium mg kg^{-1}	1009 ± 56	1461 † ± 67	692 ± 7	668 ± 15	718 ± 24	715 ± 17	656 ± 45	614 ± 24
Total Base mg kg^{-1}	3613 ± 251	5691 * ± 445	3165 ± 375	3276 ± 95	3215 ± 64	3219 ± 123	2942 ± 156	2874 ± 61
Total CEC cmol+ kg^{-1}	21.3 ± 1.1	33.3 * ± 2.2	20.2 ± 0.6	19.9 ± 0.7	17.8 ± 0.3	17.9 ± 0.7	16.3 ± 0.9	15.8 ± 0.4

Means designated * and † are significant at $p < 0.05$ and $p < 0.01$ respectively, within each year.

Table 3 Means and standard errors (n = 4) of trace metals in surface soil (0-15 cm) analyzed in 2000.

	Biodynamic	Control
Copper mg kg^{-1}	7.33 ± 0.63	6.33 ± 0.28
Manganese mg kg^{-1}	28.33 ± 2.40	21.67 ± 2.03
Iron mg kg^{-1}	80.33 ± 4.91	76.67 ± 5.70
Zinc mg kg^{-1}	1.23 ± 0.03	1.27 ± 0.12
Boron mg kg^{-1}	<0.01	<0.01

Table 4 Means and standard errors (n = 4) of biological parameters measured in soil (0-15 cm) from the biodynamically treated and untreated plots at McNab Ranch, Mendocino County, California. Fall 2002.

	Biodynamic	Control
Dehydrogenase ug TPF g^{-1} soil	2.64 ± 0.48	2.60 ± 0.24
Alkaline Phosphatase ug p-nitrophenol g^{-1} soil	110 ± 8	114 ± 9
Acid Phosphatase ug p-nitrophenol g^{-1} soil	116 ± 9	137 ± 19
Readily Mineralizable C (RMC) ug C g-^{1} soil 10 d^{-1}	956.05 ± 83.60	937.58 ± 188.00
Microbial Respiration (BR) ug C g^{-1} soil h^{-1}	31.94 ± 2.79	28.07 ± 0.39
Microbial Biomass (SIR) C g^{-1} soil	461.73 ± 17.43	441.65 ± 13.50
Nitrate - N ug g^{-1} soil	6.36 ± 0.45	6.25 ± 1.25
Ammonium - N ug g^{-1} soil	1.95 ± 0.33	1.05 ± 0.20
Aerobic N Mineralization ug NH_4^+ - N g^{-1} soil	30.25 ± 1.59	30.31 ± 2.72
Potential Nitrification ug NO_3^+ - N g^{-1} soil	154.42 ± 1.22	157.35 ± 2.04
Earthworms / m^2 in top 20 cm	267 ± 47	192 ± 3
Dehydrogenase / RMC	0.003 ± 0.001	0.003 ± 0.001
Dehydrogenase / BS	0.085 ± 0.019	0.092 ± 0.007
Dehydrogenase / SIR	0.006 ± 0.001	0.006 ± 0.000
SIR / RMC	0.490 ± 0.032	0.551 ± 0.135

Table 5 Means and standard errors (n = 4) of E4/E6, E2/E3, absorbance at 272nm, electronic transition absorbance and extinction coefficient for biodynamically treated and non biodynamically treated soils using three different extractants, NaOH, $Na_4P_2O_7$ and water.

	E4/E6	E2/E3	272nm	Electronic Transition Absorbance	Extinction Coefficient
NaOH					
Biodynamic	4.072 ± 0.055	1.176 ± 0.019	3.336 ± 0.019	7.047 ± 0.240	8.979 ± 0.750
Control	3.945 ± 0.067	1.184 ± 0.022	3.316 ± 0.015	6.857 ± 0.192	8.983 ± 0.710
$Na_4P_2O_7$					
Biodynamic	2.919 ± 0.201	1.130 ± 0.003	3.337 ± 0.010	7.664 ± 0.154	20.600 ± 1.230
Control	3.195 ± 0.199	1.128 ± 0.004	3.337 ± 0.023	7.643 ± 0.191	24.245 ± 1.290
Water					
Biodynamic	3.292 ± 0.022	1.599 ± 0.045	3.295 ± 0.007	3.870 ± 0.136	7.705 ± 1.690
Control	3.297 ± 0.012	1.477 ± 0.090	3.238 ± 0.060	4.900* ± 0.149	7.218 ± 0.170

* Indicates statistical significance at $p < 0.05$

Table 6 Means and standard errors (n = 4) of tissue analysis on second mature leaves 6th or 7th from the growth tip at veraison in 2003.

	Optimal Range	Biodynamic	Control
Nitrogen g kg^{-1}	4.9 to 15.1	21.9 ± 0.7	23.2 ± 0.5
Phosphorus g kg^{-1}	1.0 to 3.6	1.7 ± 0.1	1.7 ± 0.1
Potassium g kg^{-1}	10.0 to 20.1	7.4 ± 0.8	6.6 ± 0.5
Calcium g kg^{-1}	12.5 to 30.1	25.4 ± 0.6	25.7 ± 0.9
Magnesium g kg^{-1}	2.0 to 12.6	7.3 ± 0.5	6.9 ± 0.6
Boron mg kg^{-1}	25 to 99	36 ± 2.3	36 ± 1.7
Zinc mg kg^{-1}	15 to 52	19 ± 1.9	22 ± 5.2
Iron mg kg^{-1}	30 to 101	90 ± 2.9	107 ± 10.4
Copper mg kg^{-1}	5 to 21	3 ± 0	3 ± 0
Manganese mg kg^{-1}	60 to 201	58 ± 5.2	53 ± 1.2

Table 7. Means and standard errors (n = 4) for Enologix data (Sonoma, CA) on grapes at harvest in 2000, 2001, 2002 and 2003.

	BD	Control	BD	Control	BD	Control	BD	Control
	20 Sept 2000		22 Sept 2001		7 Oct 2002		15 Oct 2003	
Style	III	III	III	III	III	III	III	III
Index	-0.20 ± 0.23	0.70 ± 0.14	0.82 ± 0.13	0.90 ± 0.05	0.31 ± 0.08	0.47 ± 0.07	0.95 ± 0.00	0.95 ± 0.00
Ratio	0.78 ± 0.05	0.78 ± 0.04	0.85 ± 0.12	0.80 ± 0.02	0.68 ± 0.02	0.73 ± 0.02	0.95 ± 0.03	0.92 ± 0.03
Brix	24.00 ± 0.14	24.15 ± 0.10	24.87 ± 0.13	25.33 ± 0.33	26.23 ± 0.08	25.80 ± 0.21	25.88 † ± 0.09	25.55 ± 0.17
Total Phenols mg kg^{-1}	2395 ± 88	2372 ± 46	3371 ± 60	3206 ± 160	2728 ± 27	2796 ± 61	3529 * ± 37	3440 ± 35
Tannins mg kg^{-1}	1161 ± 69	1201 ± 37	1368 ± 25	1371 ± 66	1233 † ± 23	1176 ± 32	1512 * ± 16	1435 ± 19
Monomers mg kg^{-1}	318 ± 8	310 ± 0	862 ± 189	811 ± 86	510 ± 23	650 ± 63	863 ± 5	883 ± 48
Free Anthocyanins mg kg^{-1}	846 ± 25	870 ± 16	1037 ± 139	933 ± 119	903 ± 17	862 ± 19	1049 ± 16	1020 ± 20
Total Anthocyanins mg kg^{-1}	1117 ± 91	1017 ± 29	995 ± 4	983 ± 25	1108 ± 18	1092 ± 10	1337 * ± 14	1272 ± 13
Complex Anthocyanins mg kg^{-1}	199 ± 18	205 ± 15	251 ± 28	242 ± 15	188 ± 7	190 ± 5	309 ± 10	287 ± 16

Means designated * and † are significant at $p < 0.1$ and $p < 0.05$ respectively, within each year.

List of Figures

Figure 1

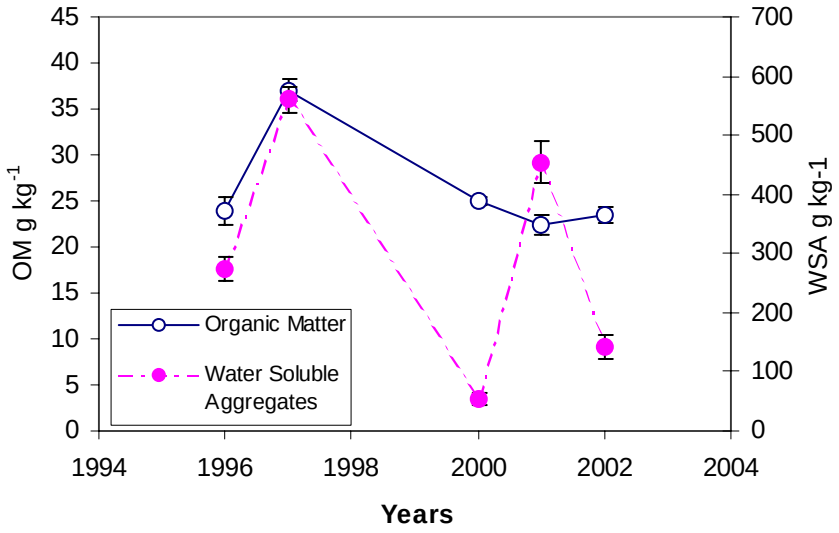


Figure 2

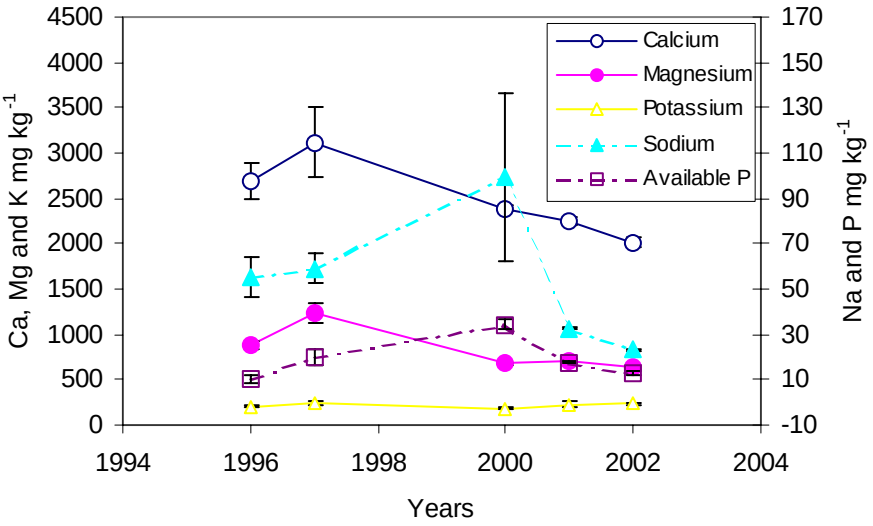


Figure 3

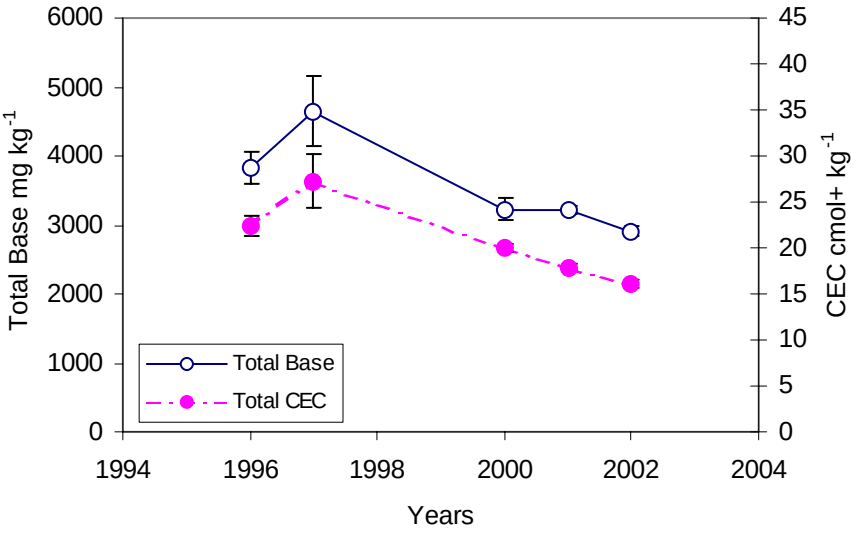
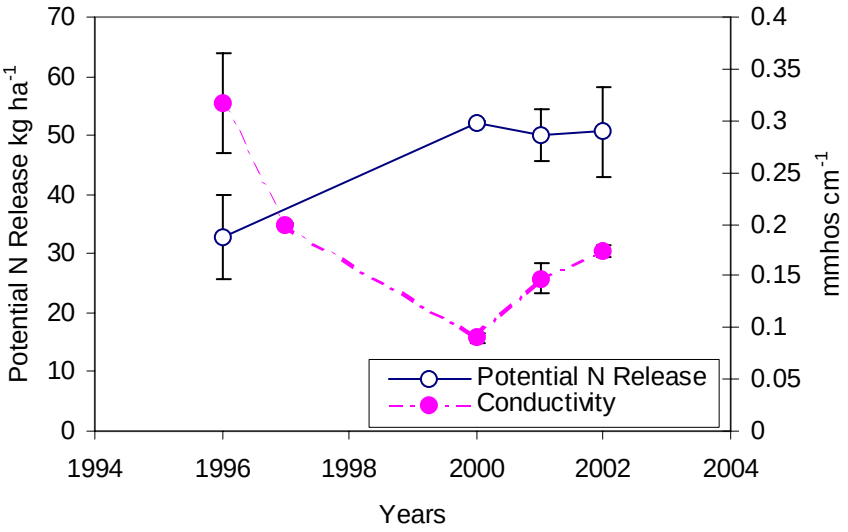


Figure 4



Biodynamic Composting of Winegrape Pomace

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(to be submitted to Compost Science and Utilization)

Biodynamic agriculture is a form of organic production that relies on compost for its source of fertilizer. Six fermented herbal substances are added to compost at the rate of 5 g each per 11 Mg in order to promote formation of a higher quality product. The effect of the biodynamic preparations on compost development was tested during on-farm composting of a grape pomace and manure mixture. Biodynamically treated compost contained 38% more ammonium and 17% higher dehydrogenase activity at the end of composting than did untreated compost in 2002. A 1 % water extract of biodynamically treated compost grew 7% taller wheat seedlings in 2002 and a 0.1 % water extract with added fertilizer grew 5 % taller wheat seedlings in 2003, than similar extracts of untreated compost. Compost in 2003 developed a high pH, lost substantial N, and supported less aerobic microbial activity. The biodynamic preparations were not shown to have an effect during these adverse composting conditions.

Introduction

Biodynamic (BD) agriculture was first presented as an ecological form of agriculture in 1924 by the Austrian philosopher Rudolf Steiner (Steiner, 1993). Since then it has gained considerable following especially in Europe, Australia and New Zealand. Biodynamics shares much in common with organic methods of farming, including soil building, crop rotations and composting. A key aspect of the BD method is the use of special preparations that are applied to the soil, crops and composts. The compost preparations consist of six fermented herbal substances, made from yarrow (*Achillea millefolium*), chamomile (*Chamomilla officinalis*), stinging nettle (*Urtica diorca*), oak bark (*Quercus robur*), dandelion (*Taraxacum officinalis*) and valerian (*Valeriana officinalis*), which are added to compost piles at the rate of 5 g each to 1 to 13 Mg of raw feedstock in order to promote the formation of a quality product (Koepf *et al*., 1990). Proponents claim that these compost preparations, by stimulating organisms already present in the substrate, produce compost that develops faster with less loss of nitrogen, fewer odor problems, and greater nutrient holding capacity (Koepf, 1993).

There has been criticism (Koepf, 1993) that past research has been biased based on nonrefereed publications and that little has been published in refereed journals. However, recent peer reviewed research showed that the compost preparations had a discernable effect on the finished product (Carpenter-Boggs *et al*., 2000). Biodynamically treated compost maintained a higher temperature throughout the active composting stage, and finished BD compost contained 65 % more nitrate, respired at a 10 % lower rate, and had higher dehydrogenase enzyme activity than untreated compost. Fatty acid analysis

indicated that BD compost had a larger proportion of bacteria to fungi than the control compost.

Boos *et al*. (1997) reported a trend towards higher initial temperature and lower respiration and ammonium in finished BD compost, indicating a faster decomposition process. Earlier research produced similar results with BD treated compost having a narrower C:N ratio, more nitrate, greater cation exchange capacity, higher respiration rate and a more even and prolonged heating period (Heinze and Breda, 1978; Ahrens, 1984; von Wistinghausen, 1984; von Wistinghausen, 1986).

Biodynamics has received attention from the wine industry in recent years with many notable wineries, particularly in France, converting to biodynamic practices (Blackburn, 1999; Meunier, 2001; Brown, 2003; Walker, 2003). This is an interesting movement in an industry concerned with quality and subtlety in its product, as biodynamic practitioners have long claimed their method leads to improved quality produce. About 500 hectares of vineyards are currently certified biodynamic in the United States with many more wine growers experimenting with the method (H. G. Courtney, 2003, The Josephine Porter Institute, personal communication). Organic winegrape production is also on the increase particularly in California where pest and disease pressures are low. Certified organic grapes now account for 1.5 % total US grape acreage (Green 2003).

Many of California's world famous wineries are using compost and other organic management techniques although they do not complete certification (Brown, 2003; Porter, 1999). Wine production creates considerable volumes of organic waste called grape pomace, which includes grape seeds, skins, and stems. Composting has become

the obvious solution for pomace disposal for many wineries. The resulting compost is often used on site to increase soil organic matter, water holding capacity and porosity.

Despite this interest, there is little in the literature concerning composting of grape pomace. General industry related articles on grape pomace composting have appeared, primarily alerting people to the possibilities that exist in this area (Logsdon, 1992; Porter, 1999; Integrated Businesses, 2000; Peterson, 2000; Brinton and York, 2003). Very little has been published in English on the physical, chemical and biological processes that occur during the process. Inbar et al. (1991) investigated organic matter transformations in grape pomace. Other studies available all involve the use of bioreactors (Streichsbeier, *et al*., 1982; Faure and Deschamps, 1990; del Rosal *et al*., 1995; Lei and VanderGheynst, 2000). This is of little use to vineyard managers composting in static piles.

This study addresses the recent interest in biodynamic viticulture and composting and is unique in that physical, chemical and biological parameters under on-farm conditions are described. The objective of the study was to test the effect of the biodynamic compost preparations on the quality of compost produced in the context of a Californian vineyard.

Materials and Methods

Composting Procedures

Feedstocks consisted of grape pomace and dairy manure with straw bedding. In March 2002 these materials were mixed 1:1 for an initial C:N ratio of 30:1 and divided into four windrows with dimensions 1.5 x 3.6 x 12.1 m. Two windrows were treated with the biodynamic preparations and two (controls) were not treated. Biodynamic treatment consisted of 5 g of each preparation numbered 502 through 507 (Koepf, 1990) each

placed in a separate hole bored 0.3 m into the pile. One set was inserted for every 10 tons of material. Preparation 507, a liquid, was lightly sprayed over the entire pile.

Piles were turned and compost samples were taken on days 0, 21, 55, 100 and 200. After turning, eight subsamples were taken along each side of the pile and thoroughly mixed together. Subsamples were stored at 4ºC prior to shipment to Woods End Research Laboratory (WERL, Mt. Vernon, ME) and Washington State University (WSU) for analysis. Temperature in each pile was recorded every 4 h with a temperature data logger (Dickson, Addison, Illinois)

This experiment was repeated in March 2003 with a further eight compost piles. Feedstocks were the same grape pomace and manure combination with dimensions of 1.5 x 3.6 x 6.1 m. The piles were turned only once on day 40 and sampling carried out as above at a depth of 60 to 90 cm, on days 0, 21, 60, 100 and 180. No water was added to the compost in 2003, as rainfall maintained adequate moisture in the piles. Temperature in 2003 was measured weekly in 8 places with a 60 cm probe (Rio Temp. Instruments, San Diego, CA)

Chemical, Physical, and Biological Analyses

The following analyses were carried out by WERL on 2002 samples. Compost was passed through a 10 mm sieve to remove any oversize material. Density was measured after TMECC, Method 03.01-A (TMECC 2002). Compost pH was measured according to EPA method 150.1 (EPA, 1983). Total Kjeldahl nitrogen was measured using EPA method 351.3 (EPA, 1983). Total carbon was measured on combustion at 550ºC. Organic matter was determined according to EPA method 160.4 (EPA, 1983). Water holding capacity (WHC) was estimated by assigning an estimate to the relative portion of organic matter (OM) and ash where: WHC = OM / 100 x 3.0 + (1-(OM / 100)) x 0.25.

The pure organic fraction (OM) is assumed to have a 300 % WHC on a dry basis and the ash (inorganic fraction) is assumed to have a 25 % WHC (W.F. Brinton, WERL, 2003, personal communication). Organic N was calculated by subtracting NH_4 and NO_3 from total N. Conductivity was measured in a saturated paste using EPA method 120.1 (EPA, 1983). Respiration rate was determined by the alkaline trapping method 05.08 B (TMECC 2002) incubated at 34ºC. Solvita maturity was measured according to method 05.08 E (TMECC 2002). Total mineral nutrients P, K, Na, Ca, Mg, Cl^-, and SO_4^{2-} were measured according to EPA Methods 202.1 to 265.3 (EPA,1983). Phytotoxicity of finished compost was tested on garden cress (*Lepidium sativum)* (adapted from Wang 1990). Analyses carried out by WERL in 2003 were density, organic matter, water holding capacity, conductivity, respiration rate and total mineral nutrients using methods above. The relative gain during composting of total N, organic matter and macronutrients was calculated by (Xdii/Xdi)/((100-Xdii)/(100-Xdi)), where X = element or parameter, di = percent concentration on d 0 and dii = percent concentration on d 200.

The remaining analyses were conducted at WSU. Moisture content on wet weight basis was determined by drying for 48 h at 65ºC. Ammonium - N and NO_3^- - N were measured in 2002 in a filtered extract of 10 g moist compost in 50 ml 0.1 M $MgSO_4$ using ion sensitive electrodes (ORION Research, Inc., Beverly, MA). In 2003, NH_4^+ - N and NO_3^- - N were measured in filtered extract from 10 g moist compost in 50 ml 1 M KCl on a Latchett QuickCHem FIA+ 8000 series autoanalyzer using the salycilate method for NH_4^+- N and the NH_4Cl_2 method for NO_3 - N. Dehydrogenase activity was measured in both years as a reduction of triphenyl tetrazolium chloride (TPF) using 1 g moist compost (Tabatabai, 1994). Alkaline phosphatase activity was measured in 2003 only using 0.25

g air-dried and ground compost (Tabatabai, 1994). All enzyme assays were analyzed on a Bio-Tek microplate autoreader model EL 311s. Nitrifying activity was measured in 2003 using a short-term incubation method modified from Schmidt and Belser (1982). Five g of moist compost was added to a solution of 100 ml 1 mM phosphate buffer, 4 ml L^{-1} 0.25M $(NH_4)_2SO_4$ and 40 mM $NaClO_4$. Nitrite - N was measured at 12, 14 and 18 h using a Latchett QuickChem FIA+ 8000 series autoanalyzer. Readily mineralizable carbon (RMC), and basal respiration (BR) were measured in 2003 using a modification of Anderson and Domsch (1978). Two g moist compost was incubated at 24ºC after moisture levels were adjusted to the wettest sample. Total CO_2 released over ten days was considered RMC, and the hourly rate of CO_2 production on day 10 was BR. Carbon dioxide in vial headspace was measured on a Shimadzu gas chromatograph model GC – 17A, with a thermal conductivity detector and a 6', 1/16'' HaySep 100/120 column. Total C and N were measured in 2003 using a Leco CNS 2000; organic N was obtained by subtracting NH_4 and NO_3 from total N.

Humic acid was extracted in 2002 using the fractionation method (Schnitzer, 1983) and purified using 0.5 % HCl- HF. In 2003 5 g dry weight equivalent compost was extracted in 0.5 M NaOH under N_2 for 24 h (Gere and Hargetai, 1970). The resulting extract was filtered (Whatman #4), diluted 1:3, neutralized to pH 8 with HCl and absorbencies measured on a Perkin Elmer Lambda 2 UV/VIS spectrometer at the following wavelengths: 665, 529, 465, 373, 365, 350, 280, 272 and 250 nm. Ratio of E4/E6 was calculated by dividing absorbance at 465 nm by absorbance at 665 nm; ratio of E2/E3 was similarly calculated from the 250 and 365 nm absorbencies. Electronic transition absorbance bands were calculated using the equation $\Delta_{ET} = 2.18(\text{In}(A_{280} / A_{350}$

))$^{-1/2}$ (Pokorná *et al*., 2001). Extinction coefficients were calculated using the equation: (Absorbance at 529 nm x extraction volume) / dry weight compost (Richter and Wistinghausen, 1981).

Growth response of wheat seedlings (*Triticum aestivum*) to aqueous compost extracts was measured in a greenhouse trial. An initial 1 : 20 extraction was made per treatment from 100 g dry weight equivalent compost in 2 L water. Samples were shaken for 30 min, rested 8 h., shaken a further 30 min and strained through a 500μm sieve. Initial extracts were diluted with water 1:5 and 1:50 to make total dilutions of 1 % and 0.1%. Wheat was soaked in water for 8 h and 2 seeds planted in each 5 x 20 cm pots containing perlite. Five ml of treatment solution was applied once per day to 10 reps (pots) per treatment. Ten treatments consisted of BD compost extract diluted 1 % plus fertilizer, BD 1 % without fertilizer, control compost extract (C) diluted 1 % plus fertilizer, C 1 % without fertilizer, BD 0.1 % plus and minus fertilizer, C 0.1 % plus and minus fertilizer, fertilizer alone, and water only. Fertilizer used was Peter's Fertilizer supplied at a rate of 25 mg kg^{-1} N, 56 mg kg^{-1} P_2O_5, 56 mg kg^{-1} K_2O, 0.14 mg kg^{-1} Mg, 0.02 mg kg^{-1} B, 0.01 mg kg^{-1} Cu chelated, 0.14 mg kg^{-1} Fe chelated, 0.07 mg kg^{-1} Mn chelated, 0.025 mg kg^{-1} Mo and 0.007 mg kg^{-1} Zn chelated. In 2002 BD 1 % compost extract contained 7.6 mg kg^{-1} N and 0.1 % extract contained 0.76 mg kg^{-1} N. Control compost 1 % extract contained 5.5 mg kg^{-1} N and 0.1 % extract contained 0.55 mg kg^{-1} N. In 2003 both BD and control compost 1 % extract contained 0.44 mg kg^{-1} N and 0.1 % extract contained 0.04 mg kg^{-1}. In 2003 two additional treatments were added, 2x and 4x original fertilizer rate. Height was recorded at 5 wk. All laboratory trials were

conducted in triplicate except humic acid extractions and nitrifying potential, which were carried out in duplicate.

Statistical Analysis

The compost experiment was analyzed as a split plot design with years as main plot and treatment as subplot. The main plot was a completely randomized design (CRD) and subplot a randomized complete block design (RCBD) with repeated measures. Test parameters measured in one year only were analyzed using a RCBD with repeated measures. Day was tested using d 0 data but treatment analysis did not include d 0. The seedling assay was designed and analyzed as a CRD with ten replicates. All statistics were measured using the SAS System for Windows Version 8 ANOVA and Fischer's Protected LSD.

All figures present 2 years of data combined unless there was a significant treatment x year or year x day interaction in which case the years are shown separately, or a significant treatment x year x day interaction when the results are presented by day. Unless specifically stated otherwise, all statistical differences represent, $p < 0.05$.

Results and Discussion

There was no statistically significant difference in temperature between the biodynamic and control compost (Figure 1). Temperature reached a high of 68ºC in 2002 and 50ºC in 2003. The large difference between years was likely due to the smaller compost piles in 2003. Both were above the 40ºC level considered to indicate a thermophilic stage and below 70ºC, which can inhibit the microbial community and cause excessive nitrogen loss. Turning operations caused temporary dips in temperature. In

2002 compost was turned on d 20, 55 and 100 and in 2003 at d 40. Turning mixes the materials, reintroduces air and increases microbial activity. The degree the pile reheats after turning is considered an indication of compost stability. In 2002 compost temperature never significantly cooled, even after 200 days of composting. Cooling was significant in 2003 ($p = 0.002$); however, final temperatures remained between 45 and 49ºC. This may indicate that the compost was not yet stable. No single parameter can be taken as a reliable measure of maturity. The lack of cooling may also have been due to insulation within the large piles and very high ambient temperature, which frequently reached 40ºC.

Density of the initial materials in 2002 was 566 kg / m^3 and increased slightly to 646 kg / m^3 by d 200. Density in 2003 was 800 kg / m^3 at the end of composting. These values fall within the ideal range recommended by WERL (2000). According to WERL, density can give a good indication of the porosity of the composting materials, which is critical to ensure adequate ventilation of the piles.

There were no significant differences in moisture content of the composts between treatments at any time or between years except on the final sampling day. Percent saturation of water holding capacity (WHC) was 92 % at d 0 and fell to 88 % by d 100 in both years. By d 200 in 2002, moisture had fallen to 62 % of WHC but in 2003 moisture was still at 86 % of WHC at day 200. Ideal moisture for maximum biological activity is 60-80 % of WHC (WERL, 2000). This suggests that these composts were somewhat wetter than optimum especially in 2003 towards the end of composting.

There was no difference in pH between treatments (Figure 2). In both years there was a small rise in pH (significant in 2003) followed by a significant drop. The drop in

pH occurred after d 20 in 2002 and after d 50 in 2003. Compost pH was significantly higher in 2003 than 2002; starting and finishing pHs were 8.1 and 7.4 in 2002 and 8.8 and 8.5 in 2003, respectively. The cause of higher pH in 2003 is unknown. Grape pomace is usually a highly acidic material, with a pH as low as 3.5 (Brinton and York, 2003). The initial pH of the grape pomace used in this study was 6.5. Compost pH usually increases during composting as organic compounds are consumed and N is mineralized (N'Dayegamiye and Isfan 1991; Frederick *et al*., 1996; Benito *et al*., 2003).

Materials that contain high initial total N frequently have excessive pH (Frederick *et al*., 1996; Charest and Beauchamp, 2002, Paredez *et al.,* 2002). In the current study total N was significantly higher in 2002; however, unlike the manure used in 2002, manure in 2003 was fresh and may have contained large amounts of urea. Charest and Beauchamp (2002) found that higher rates of urea added to paper sludge correlated with greater ammonification and high pH. Under oxygen limiting conditions, alternative electron acceptors such as nitrate are used by facultative anaerobic microorganisms in the denitrification process, which consumes protons and raises pH. Hellman *et al.* (1997) found significant methane production during composting, also a proton consuming metabolic pathway. Conversely, if conditions become anoxic, pH may decrease as acids, such as nitric acid, are formed. Compost in 2003 may have been too wet or insufficiently turned, especially towards the end of composting, promoting denitrification and pH increase.

There were no differences between treatments in total C (Figure 3), total N (Figure 2) or C:N ratios (Figure 5). In 2002 average C:N ratios started at 19:1 and dropped to 15:1. In 2003 the C:N ratio of feedstocks was 25:1 at d 0, 21:1 by d 20, then increased

again to 25:1. The rise in compost C:N in 2003 was caused by the nature of the feedstocks. The C:N ratio of the seeds (35:1) remained virtually unchanged during composting. Brinton and York (2003) showed the percentage of undecomposed grape seeds rose from 5% in the initial compost mix to more than 12% by the end of composting in 2002. Although this was not intended, the proportion of grape pomace to manure was higher in 2003. Compost contained 30 % seeds at d 0 and 40 % at d 200. When the C:N ratio of the seeds was taken into account the C:N ratio of the non seed portion in 2003 was 21:1 at d 0 and fell to 18 : 1 by d 200.

Ammonium - N was significantly higher in biodynamic compost from d 20 to d 200 in 2002 ($p = 0.02$) but treatments were not different in 2003 (Figure 6). The higher NH_4^+ - N concentration in biodynamic compost may indicate enhanced N mineralization. In both years NH_4^+ - N dropped significantly between d 0 and d 200. In 2003 NH_4^+ - N dropped after d 20 and remained very low though d 200.

There were no significant treatment effects on NO_3^--N or nitrification potential (Figures 7 and 8) although NO_3^- - N concentration was 9 % higher in the biodynamic treatment in 2002. On d 200 in 2003 all control piles and one biodynamic pile contained less than 5 mg kg^{-1} NO_3^- - N. The other three biodynamic piles contained on average 21 mg kg^{-1} NO_3^- - N, which is also considered extremely low. Carpenter-Boggs *et al*. (2000) found finished biodynamic compost to contain 65 % more NO_3^- than control compost.

Solvita Maturity, measured in 2002 only, is a quick test developed by Woods End laboratory based on release of CO_2 and NH_3^-. Index values rose over time reaching 7 by

d 200 (Figure 9), indicating the compost was fully mature. There were no differences between treatments.

When composting is successful a number of key changes in carbon and nitrogen composition occur. Percent total N and NO_3^- concentrations increase, whereas percent C and NH_4^+ decrease, leading to a decrease in the C:N ratio. In 2002 composting followed this pattern. In 2003, however, something different occurred. The very high pH, very low NH_4^+ - N and NO_3^- - N and increasing C:N ratios suggest massive N loss. Ammonia is volatilized from compost at pH 8.2 and pH in 2003 exceeded 9.0. Nitrification is also inhibited by high pH. While biodynamic preparations benefit crops under yield limiting conditions (Raupp and König, 1996) they did not ameliorate poor composting conditions in this study.

Conductivity in 2002 rose significantly over time reaching 12 mmhos cm^{-1} at d 200 (Figure 10). There was no difference between treatments. Conductivity is frequently used as an indicator of compost maturity as it is expected to increase during decomposition and then drop during the maturation phase. Conductivity higher than 10 mmhos cm^{-1} can cause soil salinity at high application levels. In 2003 conductivity only reached 3.5 mmhos cm^{-1}. reflecting the absence of NO_3^- and NH_4^+ in this compost.

There were no differences in percent P, K, Ca, Mg or Na between treatments (Table 1). Phosphorus and Na concentrations did not change over time. Potassium increased significantly from d 0 to d 200. Calcium concentration increased from d 0 to d 200 by 19 % and Mg increased by 30 %. Nutrient element concentrations increase as organic compounds are broken down and total mass declines during composting. In most cases BD compost had larger increases in cations, total N and organic matter concentration than

untreated compost (Table 1), although this difference was significant only for K ($p = 0.0855$). Potassium is the nutrient required in the highest concentrations by grapevines (Cambell and Frey 2003)

Dehydrogenase activity, as measured by the reduction of TPF, was significantly higher in biodynamic compost in 2002 ($p<0.01$, Figure 11); there was no significant difference in 2003. A large decrease over time occurred in both years. There was no difference in activity at d 0 between years, but at all subsequent sampling dates activity was substantially lower in the 2003 piles. This is further evidence that microbial activity was suppressed in the 2003 compost.

Dehydrogenase activity is frequently measured in compost studies as it is a key enzyme in the microbial oxidation of organic matter. It has been significantly correlated with a number of frequently measured parameters in composting such as respiration, water soluble C and N, nitrification potential and organic matter loss (Tiquia *et al.,* 2002; Benito *et al*., 2003). Although Fang *et al.* (1998) and others have found no correlation between dehydrogenase and microbial populations, they concede though that this test measures activity or potential activity.

Dehydrogenase activity divided by respiration is a metabolic quotient that may indicate the efficiency of functioning of the microbial community (Anderson, 1994). A higher enzyme activity coupled with lower respiration would indicate a higher metabolic efficiency in the microbial population. Similarly to Carpenter-Boggs *et al*. (2000), there was 30 % higher efficiency at d 200 in 2002 and a 19 % higher efficiency in 2003 in the biodynamic compost (Figure 12), although the differences were not statistically significant.

Alkaline phosphatase enzyme is involved in the mineralization of organic P. Phosphatase activity was analyzed in 2003 only and increased markedly during composting. There was an increasing divergence in phosphatase activity between the two treatments with time, with significantly higher phosphatase activity ($p < 0.01$) at d 100 in BD compost (Figure 13). This trend was reversed at d 200, however, with significantly higher activity ($p < 0.02$) in the control compost. Fang *et al.* (1998) showed a large drop in phosphatase activity during composting of sewage sludge as opposed to an increase over time as shown here. They also correlated lower alkaline phosphatase activities in compost with higher pH and suggested the immobilization of phosphorus could be the cause.

There were no treatment differences in respiration rate per day, readily mineralizable carbon or basal respiration. All measurements dropped significantly over time (Figures 14, 15 and 16). Respiration was significantly higher in 2003. A decline in respiration towards the end of composting is expected as microbial activity decreases and the compost enters the maturation phase. It is clear from the differences between respiration rate per day incubated at 34ºC and basal respiration at 24ºC that different methodologies can have a large effect on the actual rate measured. These inconsistencies are also evident in the literature (N'Dayegamiye and Isfan, 1991; Benito *et al*., 2003; García-Gómez *et al*., 2003).

Spectrophotometric determination of compost extracts with E4/E6 and E2/E3 ratios, absorption at 272 nm, electronic transition absorbance bands (Δ_{ET}) and extinction coefficients all showed no significant treatment effects (Figures 17, 18, 19 and 20). There

were no significant changes in E4/E6 and E2/E3 ratios over time. Absorption at 272 nm, Δ_{ET} and extinction coefficient increased significantly over time.

Chen *et al.* (1977) correlated low E4/E6 ratios to greater macromolecular size of humic substances and to lower oxygen content. A reduction in E4/E6 values as composting progresses is reported to indicate the reduction of water-soluble molecules and onset of humification processes (N'Dayegamiye and Isfan,1991). Both del Rosal *et al*. (1995) and Unsal and Ok (2001) report ratios as low as 4 for grape pomace compost. Decreasing ratios are not always seen during composting, however (Inbar *et al*., 1990; N'Dayegamiye and Isfan,1991). Decreasing E2/E3 ratios are linked to increasing aromaticity of humic substances in solution (Pokornà *et al*., 2001). Conversely increasing absorption at 272 nm and increasing Δ_{ET} are linked to increasing aromaticity (Pokornà *et al*., 2001). Data from absorption at 272 nm and Δ_{ET} indicate increasing aromaticity as compost matured. This is consistent with findings reported in the literature and represents a concentrating effect as more readily decomposable materials are broken down (Vinceslas-Akpa and Loquet, 1997; Chefetz *et al*., 1998; Inbar *et al*., 1990).

In both years wheat that received water only was shortest (Table 2). Wheat supplied with increasing levels of N was correspondingly larger. Differences in compost types occurred in 2002 at the 1 % dilution without fertilizer and in 2003 at the 0.1 % dilution with fertilizer. In both cases BD compost grew taller wheat. Interestingly, wheat growth between treatments in both years was similar despite 2003 compost containing only 5 and 7 % of the soluble N supplied by the 2002 compost. This would suggest growth promotion by compost extracts might be due to other effects as opposed to nutrient additions only. Research indicates that humic substances may cause hormone like effects

or stimulate root respiration leading to increased nutrient uptake (Day *et al*., 2000; Atiyeh *et al*., 2002). These effects are also relevant under field conditions (Day *et al.,* 2002; Zhang *et al.,* 2003).

Biodynamic agriculture has met with considerable criticism for its claims that such small quantities of substances could affect plant growth or compost development. At the dose of 5 g per preparation per 11 Mg (0.00005 %) material it is unlikely that the preparations are effective microbial inoculants. There is some evidence that microbial inoculants (at rates of 0.039 to 5 % by dry wt) speed up the initial processes in composting. These effects tend not to be maintained and additions of soil and or compost, although in substantially larger amounts (30 to 40 % by dry wt.), are just as effective (Rosal *et al*., 1995; Razvi and Kramer, 1996; Lei and VanderGheynst, 2000).

Studies, have found plant growth stimulatory substances in the biodynamic preparations. For example the field sprays 500 and 501 were found to contain high levels of cytokinins (Stearn, 1976). Goldstein and Koepf (1993) found that root length and morphology of wheat seedlings were influenced by addition of the preparations to nutrient solutions. Another study showed that the oak bark preparation 505 induced disease resistance in zucchini (Konig, 2000). Deffune and Scofield (1994) compared purchased humic acids, humic acids extracted from 500, 505 and 507, fresh 500, 505, 507 and the plant growth regulator indole-3yl-acetic acid (IAA) in nutrient solution at three dilutions. All caused a positive growth response in wheat seedlings relative to the control with humic acids most effective at 10^{-3} and the biodynamic preparations and IAA most effective at 10^{-11} and 10^{-25} .

Bacteria detect and react to extremely low levels of signal molecules in their environment, as shown in recent work on quorum sensing (Miller and Bassler, 2001). Various species of higher plants have been shown to produce signal-mimicking compounds thereby affecting bacterial density relationships (Brelles-Merino and Bedmar, 2001). Whether the preparations also contain such compounds is a possibility that has not been evaluated.

Conclusion

Grape pomace and manure compost treated with biodynamic preparations showed few significant differences compared to the same untreated compost. Most differences were observed in the first year only. In 2002 biodynamic compost had higher dehydrogenase activity, higher NH_4^+ - N concentrations. There were slightly higher increases in total N, organic matter and macronutrients in the biodynamic compost indicating less overall C loss. A 1 % water extract of biodynamically treated compost grew 7 % taller wheat seedlings than untreated compost in 2002. A 0.1 % water extract of biodynamically treated compost with added fertilizer grew 5 % taller wheat seedlings than control compost in 2003. There was higher phosphatase activity in biodynamic compost in 2003 (the only year this test was carried out). This reversed itself at day 200, however. Excessive nitrogen was lost in 2003, evidenced by increasing C:N ratios, almost complete absence of NH_4^+ and lack of NO_3^- formation. Aerobic microbial activity was also reduced. This was likely due to excessive pH in this compost. High pH could have been caused by reduced turning in 2003, excessive moisture particularly towards the end of composting, a higher proportion of grape pomace, fresher manure, or a combination of all these factors. The biodynamic preparations may influence compost development under

ideal conditions; however, they did not compensate under these adverse composting conditions.

Acknowledgements

This research was supported by Fetzer Winery, the Land Institute, and the Department of Crop and Soil Sciences at Washington State University. Special thanks go to Jim Fetzer for allowing us to initially conduct the study at McNab Ranch and to Chad Boardman for managing the field site. Many thanks to Mary Fauci, Debbi Bikfasy, Margaret Davies and Eric Harwood for much needed advice and assistance in the laboratory. And to Ron Bolton for assistance with numerous computer related problems and questions.

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TABLE 1.

Percent organic matter(OM), total nitrogen (TN), P, K, Na, Ca, Mg in compost at d 0 and d 200 at 60 to 90 cm depth (n = 6) and percent losses during composting. Negative numbers indicate an increase in concentration from d 0 to d 200.

Compost	P %	K %	Na %	Ca %	Mg %	OM	TN %	ON %
day 0								
BD	0.270	1.466	0.131	0.974	0.348	59	1.46	1.39
Control	0.293	1.566	0.130	0.964	0.320	63	1.49	1.43
day 200								
BD	0.330	1.878	0.159	1.212	0.472	52	1.44	1.41
Control	0.298	1.788	0.126	1.164	0.477	50	1.43	1.41
Change day 200 to day 0								
BD	-25%	-30%*	-20%	-28%	-37%	21%	-1%	
Control	-3%	-17%	-2%	-22%	-50%	36%	4%	

If $p < 0.1$ indicated with *

TABLE 2.
Results from wheat growth experiments in 2002 and 2003 with compost extracts of 1 % and 0.1 % dilutions. Units represent height of tallest blade in cm.

Treatment	2002	2003
BD compost		
1 %	12.64 c	11.08 e
0.1 %	9.73 e	9.18 f
Control compost		
1 %	11.72 d	11.84 e
0.1 %	9.02 e f	9.36 f
BD compost + 1 x fertilizer		
1 %	16.55 a	16.76 a
0.1 %	15.39 b	15.90 b a
Control compost + 1 x fertilizer		
1 %	16.43 a	15.78 b a
0.1 %	15.77 a b	14.60 d c
Fertilizer only		
1 x	15.01 b	13.74 d
2 x		15.53 b c
4 x		16.41 b a
Water only	8.46 f	8.42 f

Means with different letters represent significant differences at p = 0.05 within each year.

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Figure 11. Compost dehydrogenase activity, at 60 - 90 cm depth, as measured by the reduction of TPF (n = 2, 2002: n = 4, 2003). Biodynamic compost is significantly higher in 2002 (p = 0.0039).

Figure 12. Compost dehydrogenase activity ug / g / hr divided by compost respiration ug CO2-C /g / hr at 60 - 90 cm depth (n = 2, 2002: n = 4, 2003). A measure of the metabolic efficiency of the microbial population

Figure 13. Compost phosphatase enzyme activity at 60 - 90 cm depth measured as ug p-nitrophenol / g compost (n = 4, 2003).Biodynamic compost is significantly higher day 100 (p = 0.0085) and the untreated control compost at day 200 (p = 0.0171).

Figure 14. Compost respiration per day at 37 º C at 60 - 90 cm depth (n = 2, 2002: n = 4, 2003).

Figure 15. Compost readily mineralizable carbon after ten days incubation at 24 º C at 60 - 90 cm depth (n = 4, 2003).

Figure 16. Compost basal respiration per hour equilibrated for ten days at 24 º C at 60 - 90 cm depth (n = 4, 2003).

Figure 17. Compost E4/E6 and E2/E3 ratios at 60 - 90 cm depth (n = 2, 2002: n = 4, 2003).

Figure 18. Compost absorbance at 272nm at 60 - 90 cm depth (n = 2, 2002: n = 4, 2003).

Figure 19. Compost electronic transition absorbance bands at 60 - 90 cm depth (n = 4, 2003).

Figure 20. Compost extinction coefficients at 60 - 90 cm depth (n = 4, 2003).

Figure 1

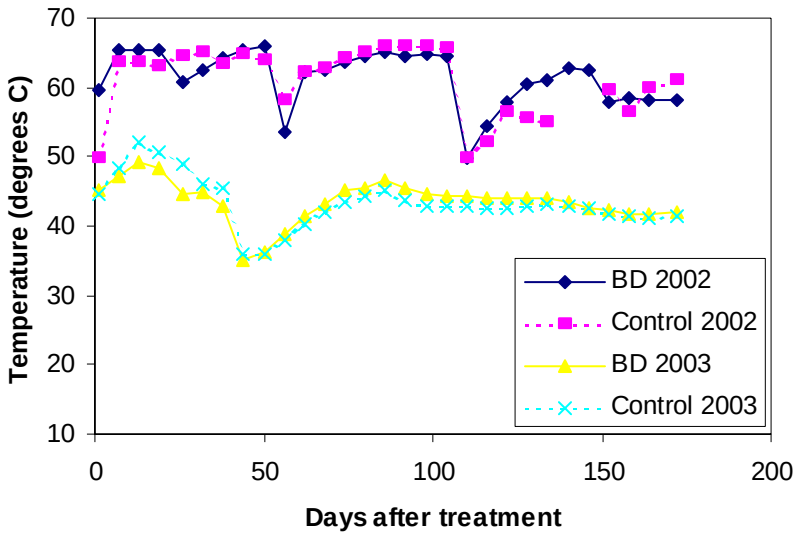
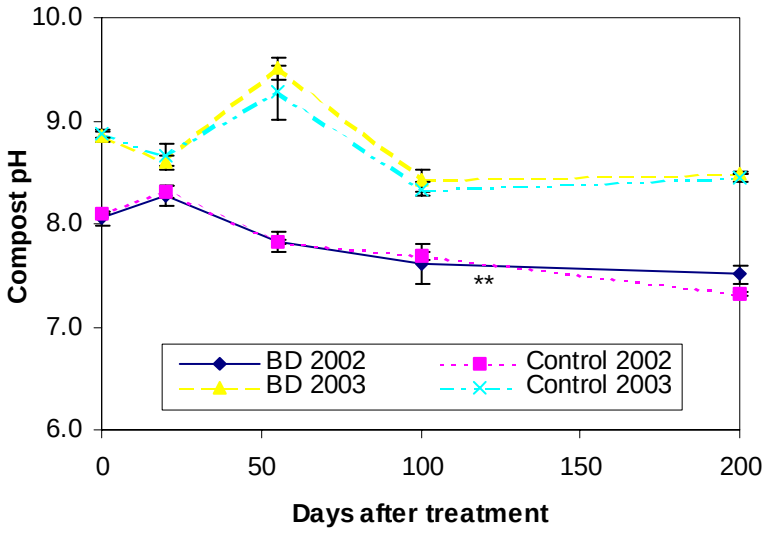


Figure 2



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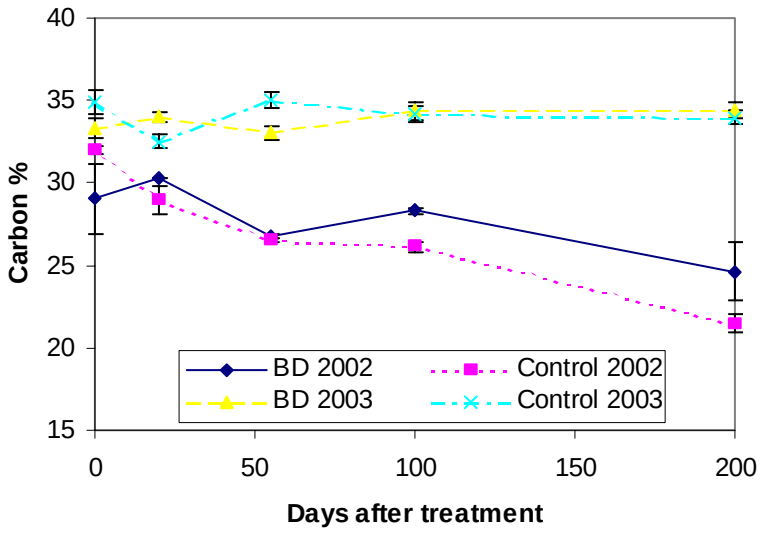

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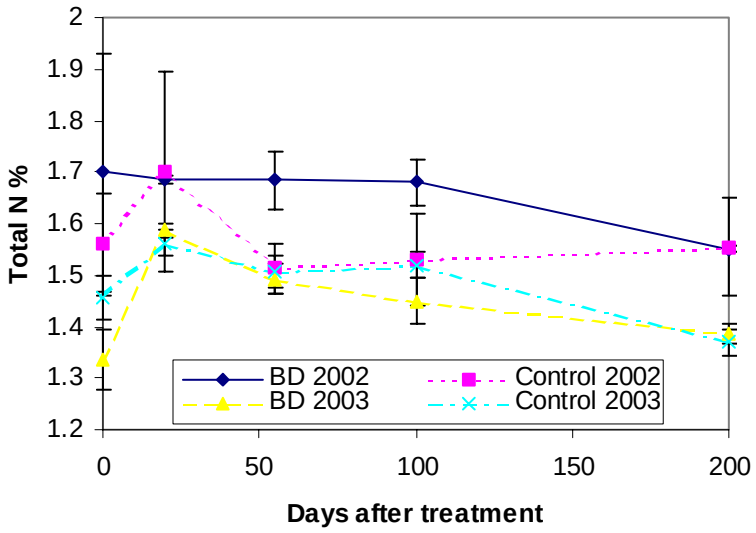

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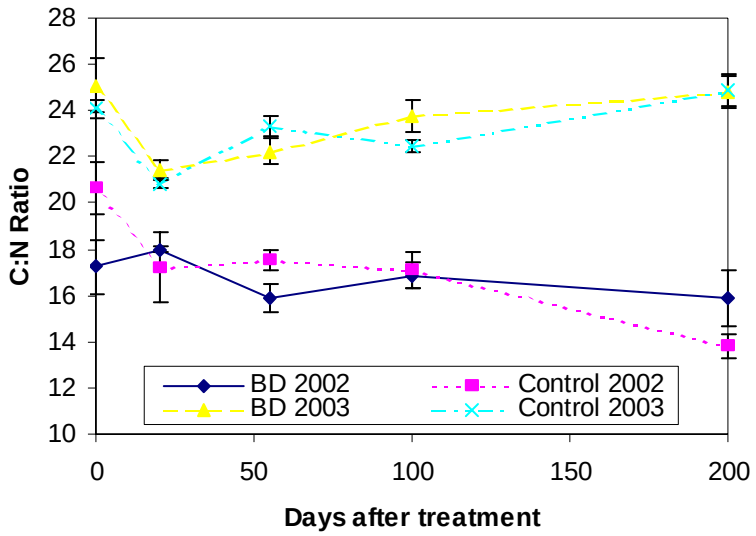


Figure 6

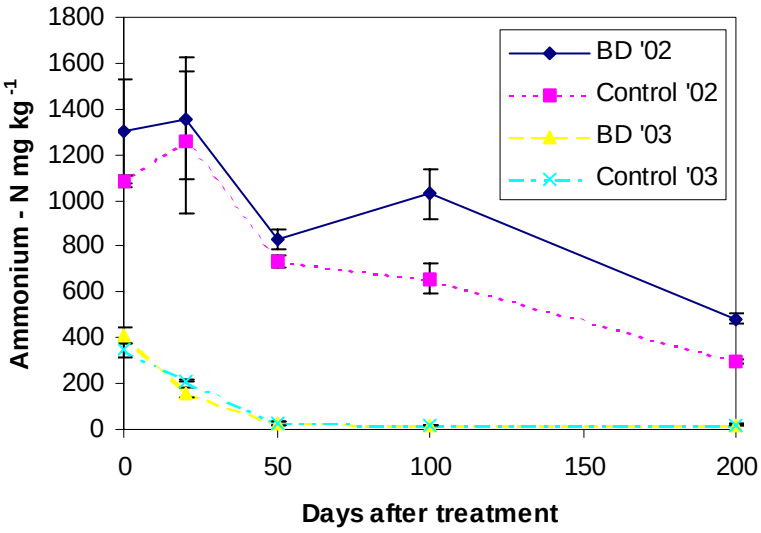


Figure 7

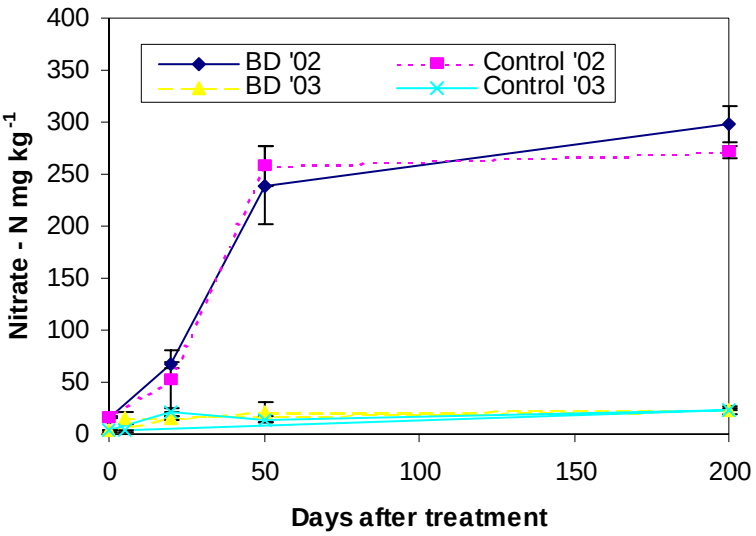


Figure 8

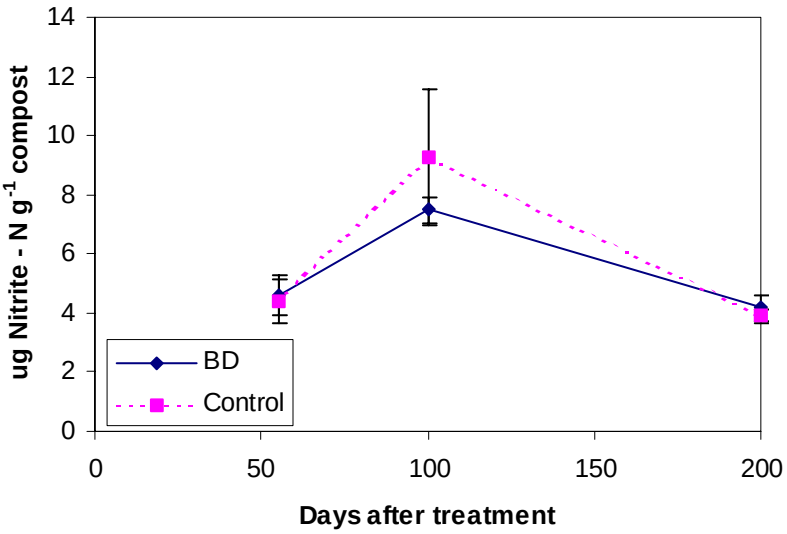
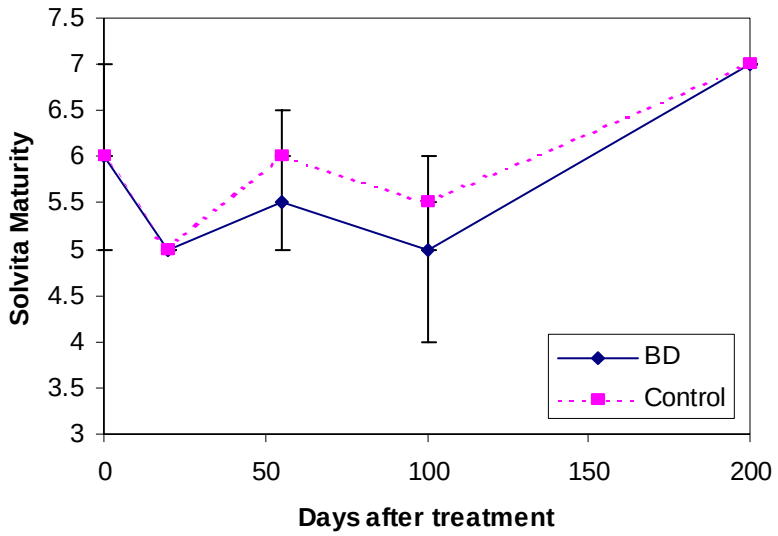


Figure 9



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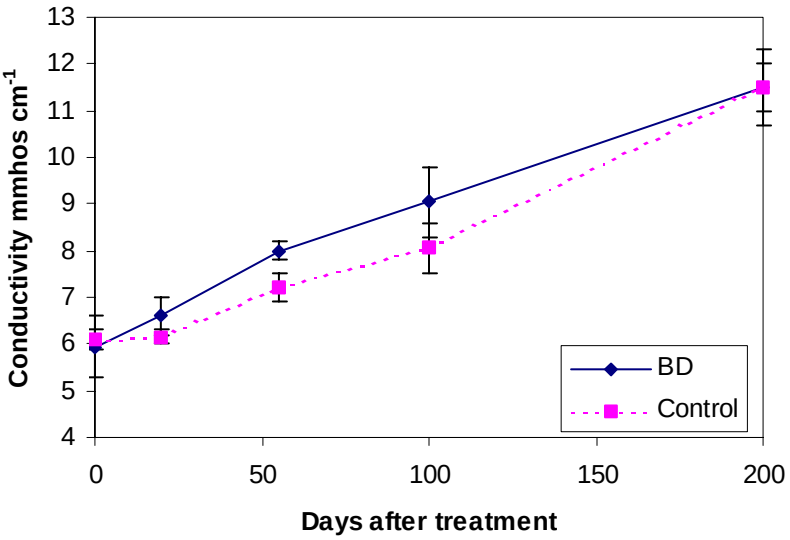

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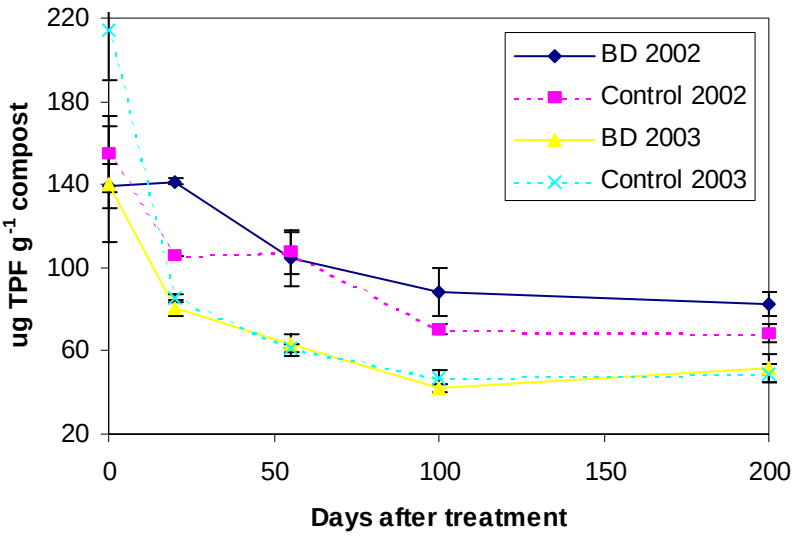

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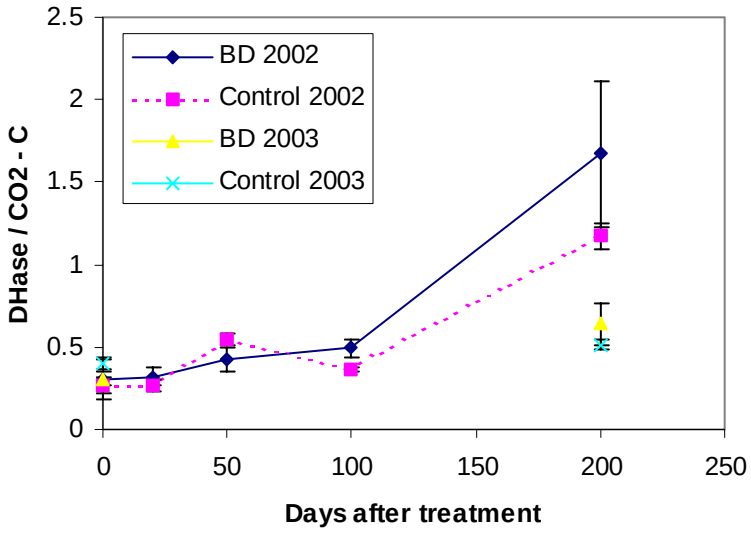

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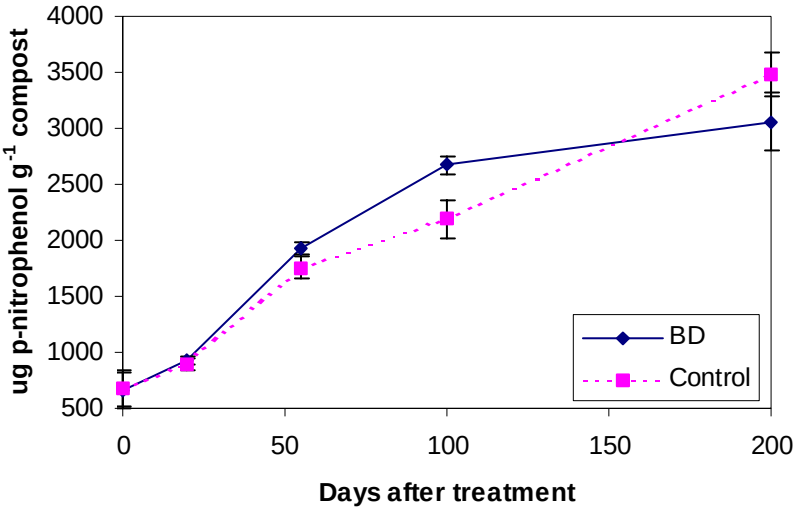

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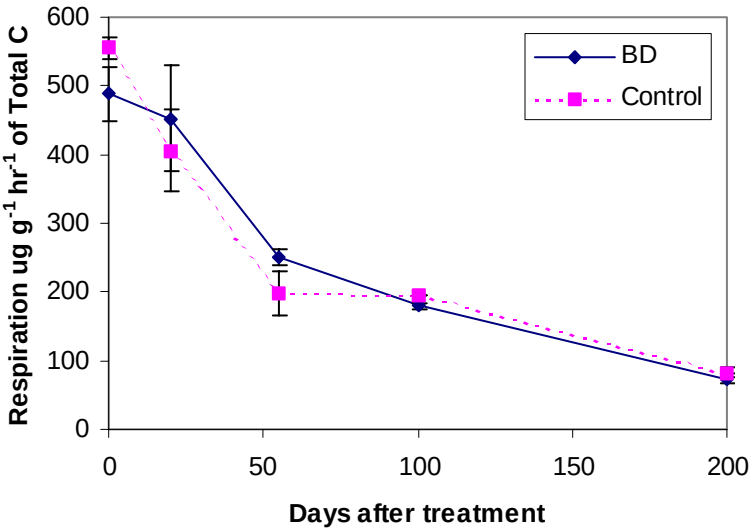

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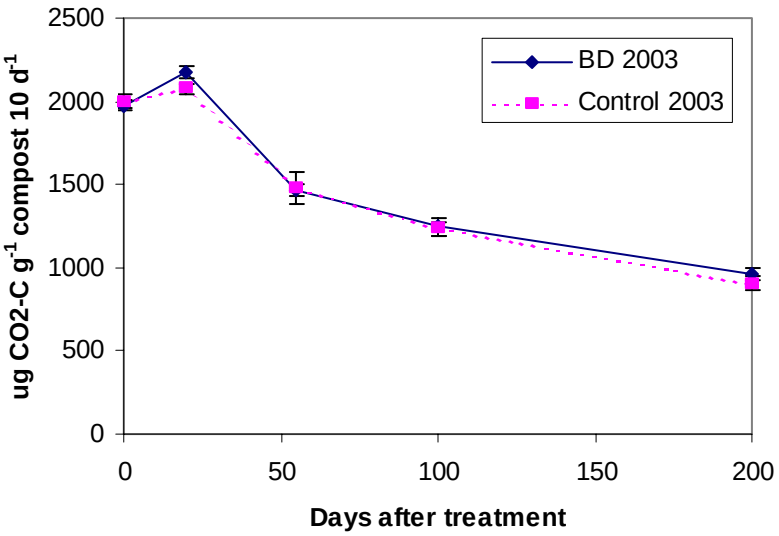

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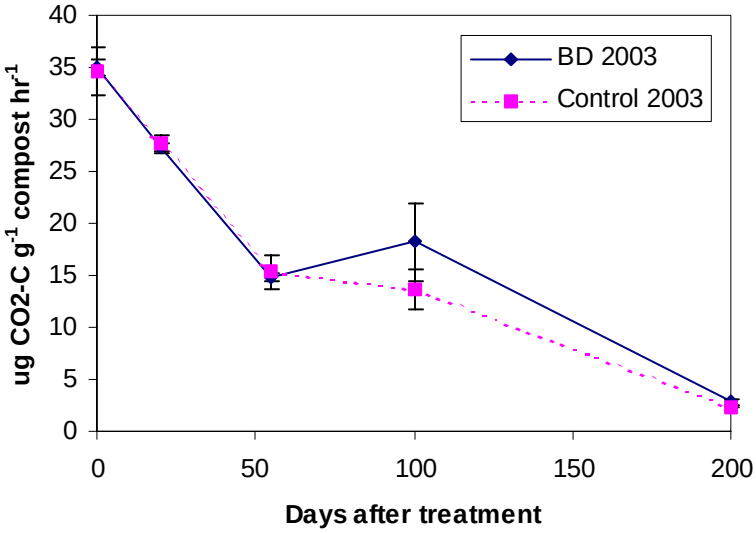

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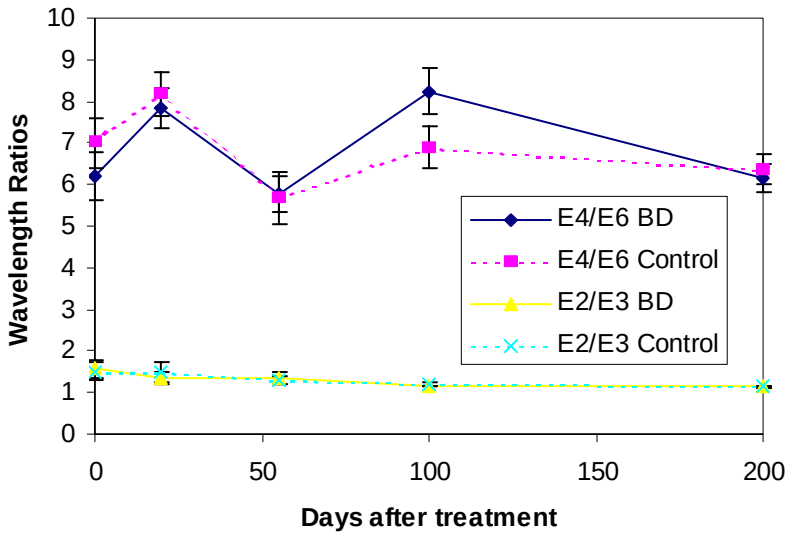

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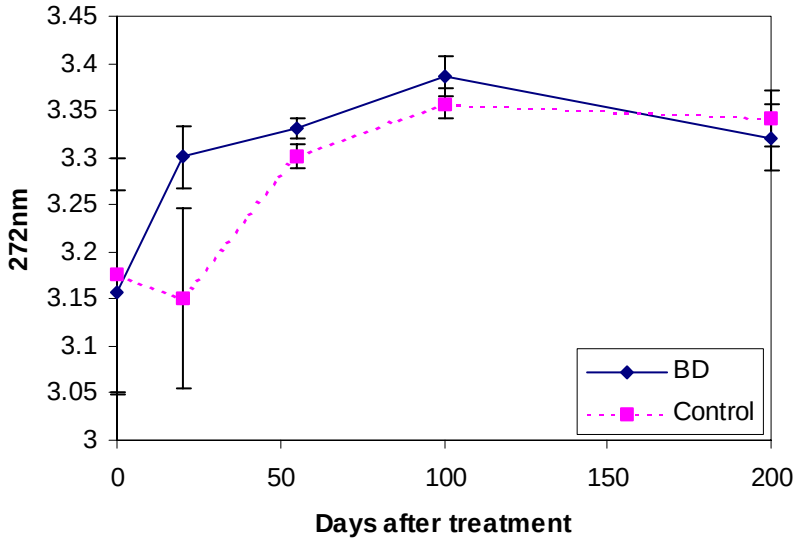

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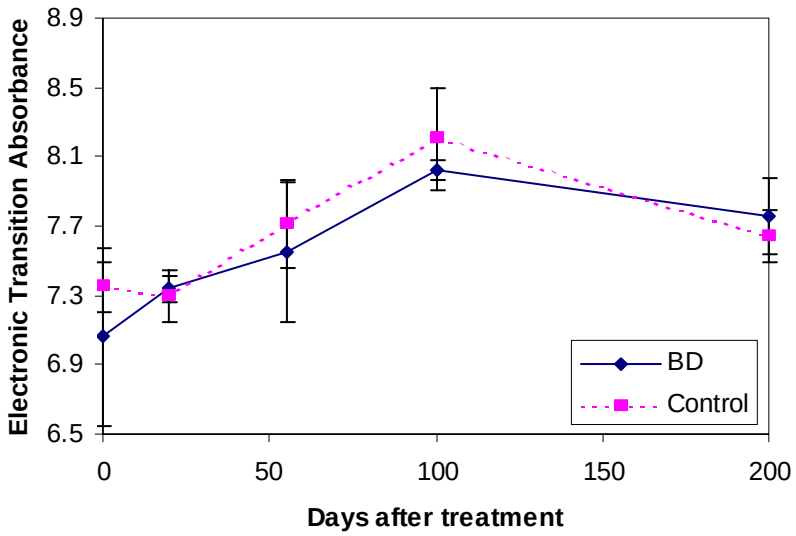

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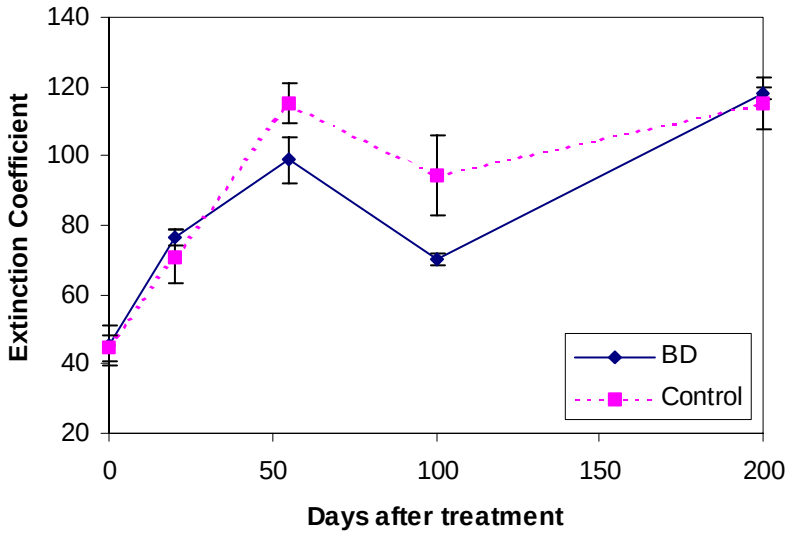

General Conclusions

There were no consistent differences in any of the soil quality parameters measured over the first six years of the experiment. When earthworms were given a choice between soil sprayed with the biodynamic preparations and unsprayed soil, however, significantly more worms migrated to the biodynamically sprayed soil. Compost was only applied once in the second year of the experiment. Instead we applied the compost preparations in the form of barrel compost spray. Compost is typically applied more regularly on biodynamic farms. Since the vines in our study were vigorous, further compost applications were not made. For growing high quality winegrapes this is a healthy practice because vines need to be stressed, somewhat, so that flavor is enhanced by recourses being allocated to the fruit and not excessive vegetative growth.

The biodynamic preparations have been shown to have larger effects under poor nutrient or soil conditions and little or no effect under good soil conditions. Soil conditions at this site were good. Effects of the single compost application could be detected for several years. Although soil mineral analysis showed the soil to be deficient in most micronutrients, these deficiencies mostly did not show up in the vine leaf petiole analysis. Since only soil in the top 15 cm was evaluated, this does not accurately reflect nutrients available to the vine as rooting depth of up to 3 meters occur where soil conditions allow.

Results of the Enologix data showed a dramatic increase in quality after 2001 when canopy management 2001 reduced the yields. Temperatures in 2002 were excessive, lowering the quality of the grapes in that year. According to the Q5 Index grapes from both treatments in 2003 received a world class rating. Biodynamically grown grapes

contained significantly higher tannins in 2002 and significantly higher Brix in 2003. Tannins, total phenols and total anthocyanins were notably higher in the biodynamic grapes in 2003. It would be interesting to see if this trend continues in the future.

Biodynamic compost was significantly higher in NH_4^+ concentration and dehydrogenase activity in the 2002. There were no differences found in the second year. The second year compost developed an excessively high pH during the early stages of composting and resulted in NH_3 loss and no significant development of NO_3^-. Why this should have happened is unclear. Compost was only turned once in the second year as opposed to three times in the first. Compost was also significantly wetter towards the end of composting than in the first year and had a higher ratio of grape pomace to manure. The manure used in the second year was fresher and may have contained large amounts of urea. Overall, it was shown that the biodynamic compost preparations had some effects on compost development under ideal conditions, but had almost no effect under poor compost conditions. Although biodynamic preparations have been shown in other studies to benefit crops under yield limiting conditions, they did not ameliorate poor composting conditions in this study.

Wheat seedlings grown in 1 % extract of biodynamically treated compost were significantly taller in 2002 than in untreated compost. Wheat seedlings grown in 0.1 % extract of biodynamically treated compost with added fertilizer were significantly taller in 2003 than in untreated compost with added fertilizer. Even though 2003 compost contained 5 and 8 % of the soluble N contained in 2002 compost, growth response between years was not different indicating additional effects to nutrients might be occurring. Research has shown compost may enhance plant growth by hormonal effects

and/or by enhancing nutrient uptake. This is of particular interest in the context of organic and biodynamic agriculture. If the efficiency in the way plants can access nutrients is enhanced and growth stimulation is achieved by the addition of organic fertilizers in the form of composts, vermicomposts and compost teas, this would be an important discovery in the quest for a more energy efficient and sustainable agriculture.

This study only scratches the surface of the question as to whether the biodynamic preparations affect grape and wine quality. The next step will be to continue to monitor grape quality and to make and evaluate wine made from biodynamically and nonbiodynamically grown grapes. Other questions in need of further study include looking at differences in root structure and rooting depth between the two treatments, and evaluating the role of microbial metabolites and humic substances on root development, nutrient uptake and composition of grapes. The question of whether soil has any direct influence on the flavor and complexity of wines, and if so, by what means is a controversial subject in itself and in need of more study.

More research is needed to confirm whether or not the preparations produce a measurable effect on winegrape quality and, if they do, to pursue the exact mechanisms. Based on the results in this study, the use of biological organisms as indicators may be the most fruitful approach. When physical, chemical and biological analyses failed to show significant differences, effects in plant growth response and earthworm behavior were nevertheless demonstrated, possibly indicating real effects of as yet undetermined biological significance. I believe that practices appearing at first glance to be outlandish can provide valuable stimulation in the pursuit of new discoveries in science.

Appendix

Table 1 Means and standard errors from initial soil samples taken at the start of the experiment in 1996 at three depths, 0-15 cm, 15-30 cm and 30-45 cm from biodynamic and control plots before treatments were applied.

	BD 0-15 cm	**Control 0-15 cm**	**BD 15-30cm**	**Control 15-30cm**	**BD 30-45cm**	**Control 30-45cm**
pH in H2O	6.9 ± 0.10	7.0 ± 0.07	6.8 ± 0.05	6.9 ± 0.02	6.9 ± 0.00	6.8 ± 0.06
pH in CaCl2	6.8 ± 0.08	6.8 ± 0.05	6.6 ± 0.05	6.8 ± 0.03	7.6 ± 0.91	6.7 ± 0.17
Organic Matter g kg^{-1}	24 ± 1.5	22 ± 1.2	22 ± 5.2	24 ± 3.1	20 ± 1.7	18 ± 2.0
Biological Respiration g kg^{-1} C wk^{-1}	15.0 ± 3.0	11.0 ± 5.1	10.0 ± 3.8	12.0 ± 4.9	9.0 ± 3.6	13.0 ± 4.6
Total CO_2 Output mg $kg^{-1}wk^{-1}$	715 ± 162	512 ± 243	500 ± 244	589 ± 265	369 ± 161	488 ± 209
Potential N Release kg ha^{-1}	40.3 ± 8.3	29.1 ± 12.4	28.3 ± 12.4	33.3 ± 13.5	20.9 ± 8.2	27.7 ± 10.7
Water Soluble Aggregates g kg^{-1}	250 ± 30	233 ± 24	210 ± 21	300 ± 21	290 ± 29	217 ± 22
Conductivity mmhos cm^{-1}	0.4 ± 0.07	0.2 ± 0.00	0.2 ± 0.00	0.3 ± 0.07	0.2 ± 0.03	0.2 ± 0.09
Available Phosphorus mg kg^{-1}	10.7 ± 2.7	8.0 ± 0.0	8.0 ± 0.0	10.3 ± 2.3	10.7 ± 2.7	8.0 ± 0.0
Nitrate mg kg^{-1}	30.7 ± 8.2	12.0 ± 1.5	15.0 ± 2.0	33.3 ± 6.8	20.0 ± 9.1	16.0 ± 4.5
Reserve Phosphorus mg kg^{-1}	18.7 ± 4.7	11.0 ± 3.0	12.3 ± 3.0	24.3 ± 2.8	17.7 ± 8.7	13.3 ± 5.3

Chloride mg kg^{-1}	4.7 ± 0.9	3.0 ± 0.6	11.0 ± 6.7	4.7 ± 0.7	4.3 ± 0.3	7.33 ± 2.8
Sulphate mg kg^{-1}	32.3 ± 23.3	4.0 ± 1.0	5.7 ± 3.7	6.0 ± 1.5	6.3 ± 2.4	3.7 ± 0.3
Potassium mg kg^{-1}	194 ± 25	210 ± 53	147 ± 31	208 ± 12	183 ± 25	163 ± 14
Sodium mg kg^{-1}	52 ± 12	62 ± 20	67 ± 23	58 ± 14	54 ± 16	54 ± 15
Calcium mg kg^{-1}	2889 ± 386	2938 ± 320	227 ± 346	2491 ± 157	2259 ± 401	2193 ± 135
Magnesium mg kg^{-1}	925 ± 66	1168 ± 211	1138 ± 139	839 ± 46	930 ± 179	1041 ± 42
Total Base mg kg^{-1}	4061 ± 409	4378 ± 601	3623 ± 528	3597 ± 164	3427 ± 597	3451 ± 184
Total CEC cmol+ kg^{-1}	23.9 ± 1.7	25.9 ± 3.8	22.5 ± 3.3	20.9 ± 0.7	21.1 ± 3.6	22.3 ± 1.5

Table 2 Worm behavior raw data: Actual numbers of worms counted in each section were converted to % of total population for analysis.

Soil Treated with Biodynamic Sprays		The Same Soil Untreated	
Actual numbers	% population*	Actual numbers	% population
48	92	4	8
25	71	10	29
16	80	4	20
23	45	28	55
31	89	4	11
22	54	19	46
23	43	30	57
32	84	6	16
34	74	12	26

* indicates statistical significance at $p < 0.05$.