

Effects of agricultural biostimulants on soil microbial activity and nitrogen dynamics

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Received 28 February 2000; received in revised form 19 December 2001; accepted 20 December 2001

Abstract

We investigated the effects of two commercially available soil biostimulants, designated Z93 and W91, on key microbial and nutrient cycling processes in the soil, by conducting short-term (1 week) and longer-term (8 weeks) soil incubations in the laboratory. In the short-term soil incubations, the two compounds differed in their effects on microbial activity: Z93 was effective over a wide range, stimulating substrate-induced respiration (SIR) and dehydrogenase activity (DHA) at remarkably low concentrations (0.5–500 nl/g soil); W91 stimulated SIR at these concentrations, but also inhibited DHA. In longer-term soil incubations, we amended batches of soil with either finely-ground alfalfa leaves, wheat straw, or added no amendments, to alter patterns of soil nitrogen mineralization and immobilization. We treated these soils with Z93 and W91 at two concentrations (0.005 and 0.5 μ l/g soil), and incubated them for up to 8 weeks. These extremely low doses of both Z93 and W91 influenced soil SIR, DHA, and cellulase activity significantly ($P < 0.05$). Both compounds also influenced soil nitrogen dynamics significantly; the extent depending upon the quality of the organic amendments. In the alfalfa-amended soil there was a steep increase in $\text{NO}_3\text{-N}$ concentration during the incubation due to the rapid mineralization of nitrogen-rich alfalfa material. However, in this soil, both Z93 and W91 reduced $\text{NO}_3\text{-N}$ concentrations greatly after 56 days. In the straw-amended soil, mineral nitrogen concentrations were very low, probably due to rapid immobilization of nitrogen by microbial biomass. In this soil, treatment with both compounds decreased microbial biomass nitrogen and increased dissolved organic nitrogen (DON), relative to that in the controls. Our results suggest that the two biostimulants can stimulate both the breakdown and mineralization of soil organic materials, perhaps by selectively inhibiting or stimulating particular components of the microbial community, leading to lasting (8 weeks or longer) increases in soil nitrogen availability. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Biostimulants; Microbial activity; Nitrogen dynamics; Soil enzymes

1. Introduction

There are numerous products currently available for agricultural use which fall into the broad category of ‘soil biostimulants’ (Miller, 1990). They are

claimed to enhance crop growth and yield through a widely varying mechanisms including: inoculation of soil with microorganisms, activation of soil microbial activity, promotion or augmentation of the activities of critical soil enzymes or plant growth hormones and supplementation with micronutrients. For the most part, most of these products have been dismissed by soil scientists and agronomists as largely ineffective, although, it has been sometimes conceded that there

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may be a valid scientific basis for their use (Miller, 1990). In fact, relatively little research has been done to document the effects (or non-effects) of many biostimulants on crop production, or to provide evidence of their potential effects on soil processes.

Two soil biostimulants, one of which is widely marketed in USA, (herein designated Z93 and W91) have been reported to enhance the yields of many crops at remarkably low application rates, typically 0.2–1.1 l/ha (Ag Spectrum Co., 1996). These compounds, developed by Ag Spectrum Co. (DeWitt, Iowa), comprise solutions of fermentation products and trace minerals. Due to the proprietary nature of the products that we investigated, we cannot present detailed information on their overall composition, although, we can supply data on their elemental composition (Table 1). Although, their beneficial effects on crop yields at such low dosage rates have been fully documented in numerous field trials of cereals and other crops (AgroPlus Inc., 1991, 1992; Ag Spectrum Co., 1996), their exact mode of action is currently unknown.

We made initial preliminary investigations into the effects of the biostimulants Z93 and W91 on soil

microbial activity and nitrogen dynamics. Specifically, our objectives were to characterize the effects of these compounds on soil microbial biomass and respiratory/degradative activity, as well as on nitrogen pools and transformations in the soil. We first developed short-term dose-response relationships for these compounds to determine their active ranges of concentration. We have since conducted longer-term soil incubations to investigate the effects of these compounds in soils with widely varying organic matter quality and nitrogen mineralization/immobilization dynamics (Chen et al., 2002).

2. Methods

2.1. Dose-response relationships

Samples of Z93 and W91 were obtained from Ag Spectrum Company (DeWitt, Iowa). Z93 is marketed under the trade name GroZyme[®], W91 is an experimental product not yet marketed. Both compounds were applied separately to small batches of soil (~50 g) in 150 ml plastic containers in the laboratory. Two soils were used for this study, a Huntingdon silt-loam (mesic Flueventic Hapludoll) collected from the top 20 cm surface of soil in agricultural research plots in Piketon, Ohio, and a Canfield silt-loam (fine, mixed, mesic Fragiudalf) from Wooster, Ohio. Both soils had been air-dried and stored for about 1 year before being sieved through a 2 mm mesh, to remove organic debris and gravel, and to destroy large aggregates. The biostimulant concentrations used ranged from 0 (control) to 50 µl/g soil. Typical field application rates are 0.2–1.1 l/ha or about 0.005 µl/g soil. Soil water content in the containers was maintained at ~20% (dry weight basis). The containers were loosely capped and incubated for 1, 3, or 7 days at 30 °C, after which soil microbial respiration and dehydrogenase enzyme activity were measured. For each soil, there were four replicate containers for each chemical concentration.

Substrate-induced respiration (SIR) was determined by adding 5 ml of glucose solution (32 mg/ml) to 25 g subsamples of soil and placing them in 11 jars for 6 h. The carbon dioxide (CO₂) respired during this period, was trapped in 20 ml 0.02M NaOH, and subsequently measured by titration with HCl to

Table 1
Elemental composition of the compounds Z93 and W91 (mg/l in solution)

Element	Z93	W91
C ^a	818	16514
N ^a	21	1203
P ^b	859	232
K ^b	62	1200
Ca ^c	nd	nd ^d
Mg ^b	725	16
S ^b	nd	nd
Fe ^c	Trace	nd
B ^b	Trace	nd
Mn ^b	621	1
Zn ^b	819	16
Cu ^b	716	70
Mo ^c	nd	nd
Co ^c	Trace	nd
Si ^c	nd	nd
Ba ^c	nd	Trace
Se ^b	74	10
Al ^c	Trace	nd
Total solids (mg/l)	13140	74591

^a Determined using a C:N analyzer.

^b Determined by ICP.

^c Determined by mass spectrometry.

^d Not detected: nd.

Table 2

Results of *F*-tests from an overall ANOVA for SIR, DHA, cellulase enzyme activity, and concentrations of microbial biomass N (BION), NH₄-N, NO₃-N, and DON in effects of biostimulants

Source of variation	SIR	DHA	Cellulase	BION	NH ₄ -N	NO ₃ -N	DON
MODEL	0.001**	0.001**	0.001**	0.001**	0.001**	0.001**	0.001**
<i>R</i> ²	(0.91)	(0.92)	(0.92)	(0.83)	(0.85)	(0.96)	(0.86)
Biostimulant	0.001**	0.002**	0.533	0.006**	0.042*	0.238	0.001**
Amendment	0.001**	0.001**	0.001**	0.001**	0.001**	0.001**	0.001**
Date	0.001**	0.001**	0.001**	0.016*	0.001**	0.001**	0.001**
Biostimulant × date	0.001**	0.319	0.644	0.074	0.001**	0.977	0.001**
Biostimulant × amendment	0.006**	0.049*	0.113	0.060	0.048*	0.068	0.001**
Amendment × date	0.001**	0.001**	0.001**	0.001**	0.001**	0.001**	0.001**
Biostimulant × amendment × date	0.001**	0.028*	0.114	0.046*	0.001**	0.915	0.001**
Concentration (biostimulant)	0.831	0.242	0.093	0.029*	0.006**	0.575	0.304
Concentration (biostimulant) × date	0.105	0.001**	0.643	0.015*	0.501	0.709	0.528
Concentration (biostimulant) × amendment	0.241	0.032*	0.341	0.263	0.167	0.354	0.430
Concentration (biostimulant) × amendment × date	0.062	0.045*	0.035*	0.617	0.001**	0.998	0.945

* *P* < 0.05.

** *P* < 0.01.

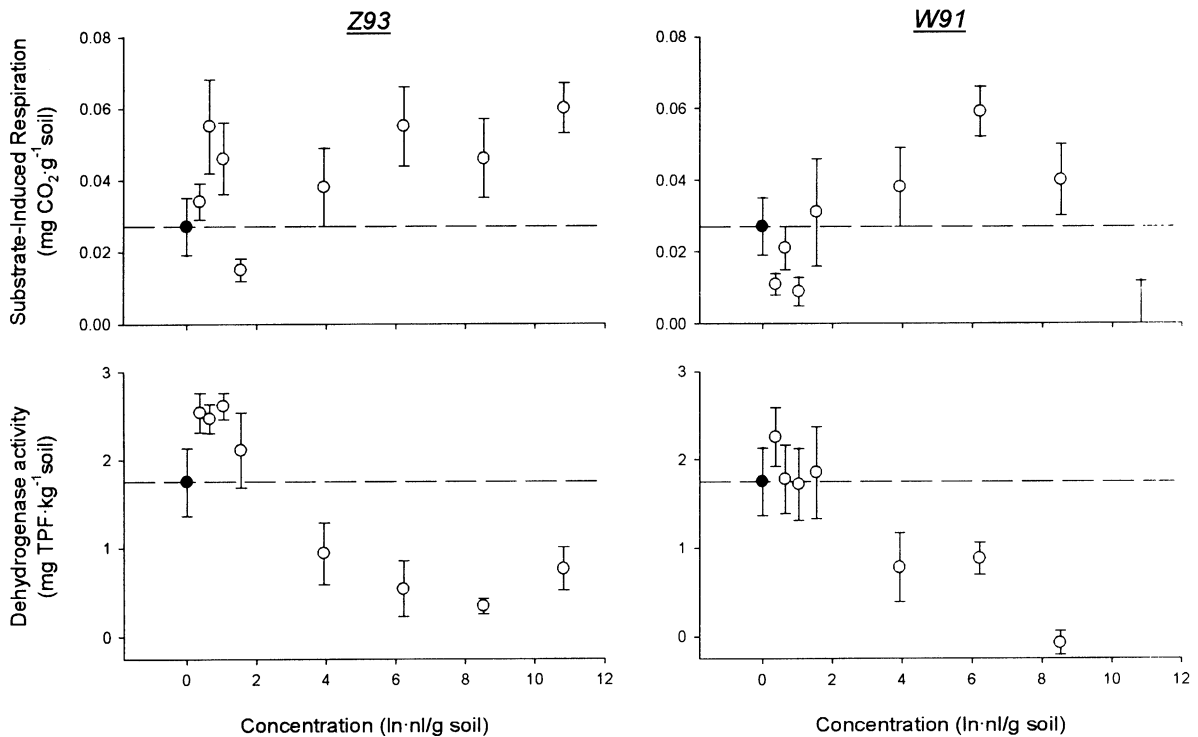


Fig. 1. Representative dose-response relationships for microbial respiration and DHA in Huntingdon silt-loam soil 1 week after treatment with varying concentrations of the biostimulants Z93 and W91 (mean ± S.E.). Solid circles and dashed lines indicate untreated (control) responses.

a phenolphthalein endpoint after adding excess BaCl_2 (Anderson, 1982). Soil dehydrogenase activity (DHA) was measured using a modification of the method of Casida (1977). One gram of freshly-sieved soil was incubated at 40°C for 6 h in test tubes containing 1 ml 0.5% 2,3,5-triphenyltetrazolium chloride in 0.5 M Tris buffer (pH 7.6); accumulation of the end-product triphenyl formazan (TPF) was determined in methanol extracts (10 ml) using a Lachat flow-injection autoanalyzer with a 480 nm filter.

2.2. Eight-week soil incubations

To investigate the effects of the two biostimulants on biological activity, in soils differing widely in nitrogen availability and rates of N mineralization

and immobilization, different batches of the Canfield silt-loam soil were amended with either alfalfa leaves or wheat straw, or left unamended. The organic amendments were ground finely, passed through a 0.2 mm mesh and mixed into the soil at a ratio of 50:1 (soil:amendment, dry weight basis) prior to the application of the biostimulants.

Soils were treated with either one or the other of two biostimulants each at two concentrations or left untreated. The 'low' concentration was equivalent to the recommended field application rates ($0.005 \mu\text{l/g}$ soil), based on the assumption that the compounds would be incorporated into the surface 2 cm of soil, with a bulk density of approximately 1.2 g/cm^3 . The 'high' concentration was 100-fold greater ($0.5 \mu\text{l/g}$ soil). Appropriate quantities of biostimulant-containing soil

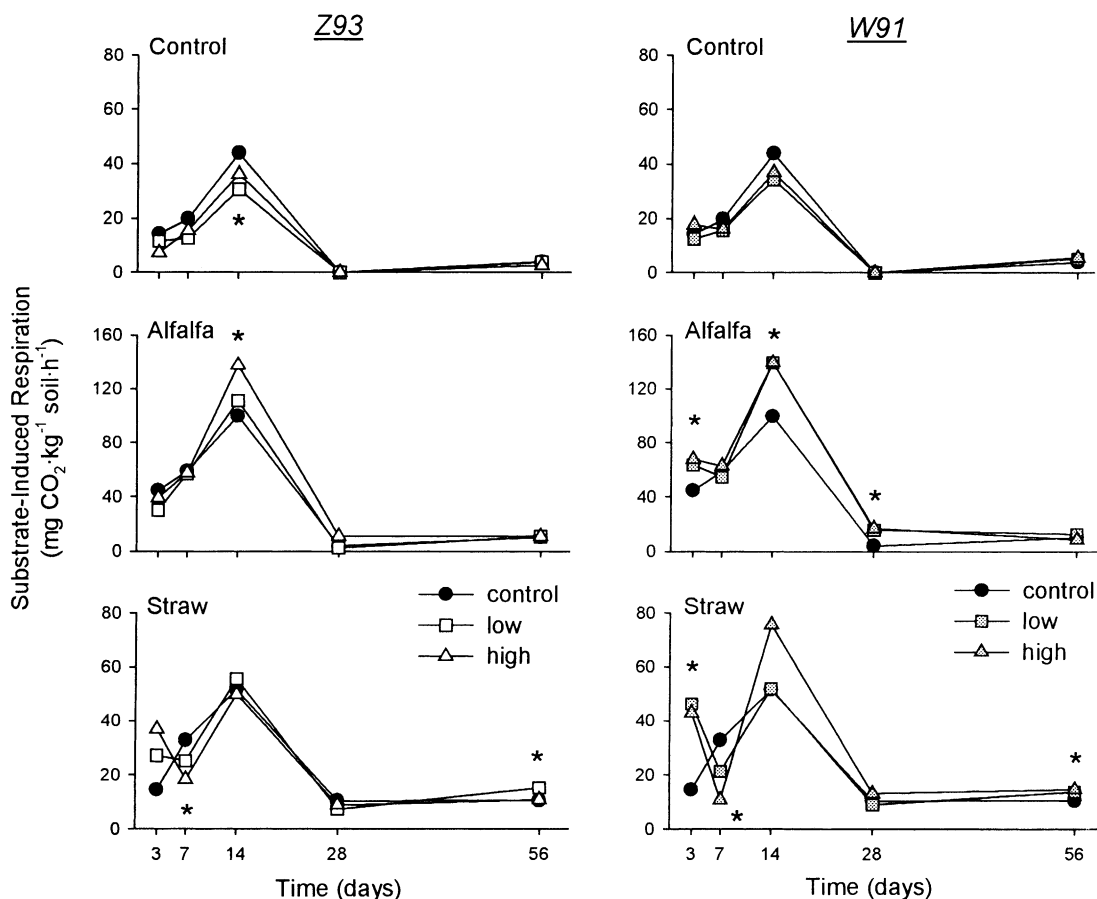


Fig. 2. SIR of soils treated with Z93 and W91 at two concentrations (mean \pm S.E.). Symbols indicate significant chemical effects for each date at $P < 0.05$ (*).

utions were mixed thoroughly into batches of soil, so that the soils were moist but not saturated (~20% soil water content, dry weight basis). Equal amounts of deionized water were added to the soils every few days to maintain constant moisture. Small disks of cellulose filter paper were placed in the bottom of the containers to measure rates of cellulose decomposition (cellulase enzyme activity).

After periods of 3, 7, 14, 28, and 56 days subsequent to biostimulant application, soils from four replicate microcosms of each treatment combination were assayed for microbial activity (SIR, dehydrogenase enzyme activity, and cellulase enzyme activity), and nitrogen concentration (ammonium-N, nitrate-N, dissolved organic N, microbial biomass N). SIR and

DHA were measured as described above. Cellulase activity was measured by removing the cellulose filter disks from the containers, drying at 60 °C, and weighing the bottom disk, after discarding the top disk that was in contact with the soil. Soil mineral N concentrations (NH₄-N and NO₃-N) were determined in 0.5M K₂SO₄ extracts (1:5 soil to extractant) using a Lachat QuikChem AE flow-injection autoanalyzer. Dissolved organic nitrogen (DON) was calculated as the difference between the initial mineral nitrogen concentration and the NO₃-N concentration determined after alkaline persulfate digestion of the soil extracts (Cabrera and Beare, 1993). Microbial biomass nitrogen was determined using the chloroform fumigation-direct extraction method (Brookes et al., 1989).

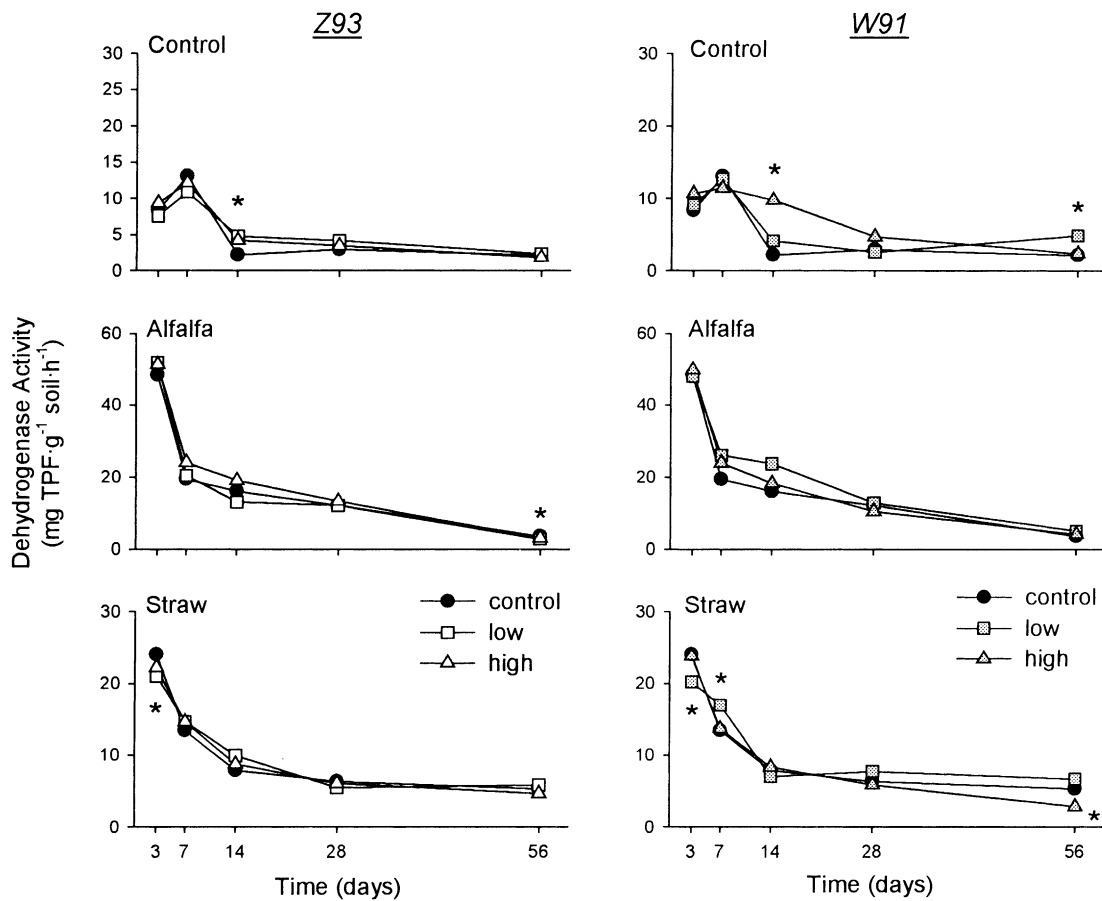


Fig. 3. Dehydrogenase enzyme activity of soils treated with Z93 and W91 at two concentrations (mean ± S.E.). Symbols indicate significant chemical effects for each date at $P < 0.05$ (*).

2.3. Statistical analyses

We used analysis of variance (ANOVA) to test the significance of differences among means of the dependent variables measured in the soil incubations. Initially, an overall ANOVA model was used for each dependent variable; the model included the main effects of date, soil amendment, and biostimulant treatment, and their two- and three-way interactions. Additionally, biostimulant concentrations were nested within each biostimulant treatment. If biostimulant treatment main effects or interactions were significant ($P < 0.05$), then the appropriate ANOVAs were calculated by individual date and amendment to determine the significance of the biostimulant effects, relative to the untreated controls, using linear contrasts (planned

comparisons) ($P < 0.05$ or otherwise indicated). Data were rank-transformed before analyses to equalize error variances and, given the small sample sizes, to provide a potentially more robust method than traditional parametric methods (Conover and Iman, 1981). Table 2 summarizes the results of the overall ANOVA.

3. Results and discussion

3.1. Dose-response relationships

The responses in soil microbial respiration and DHA, 1 week after soil treatment with Z93 and W91, at widely varying concentrations, are illustrated for the Huntingdon soil in Fig. 1. Results for the Canfield

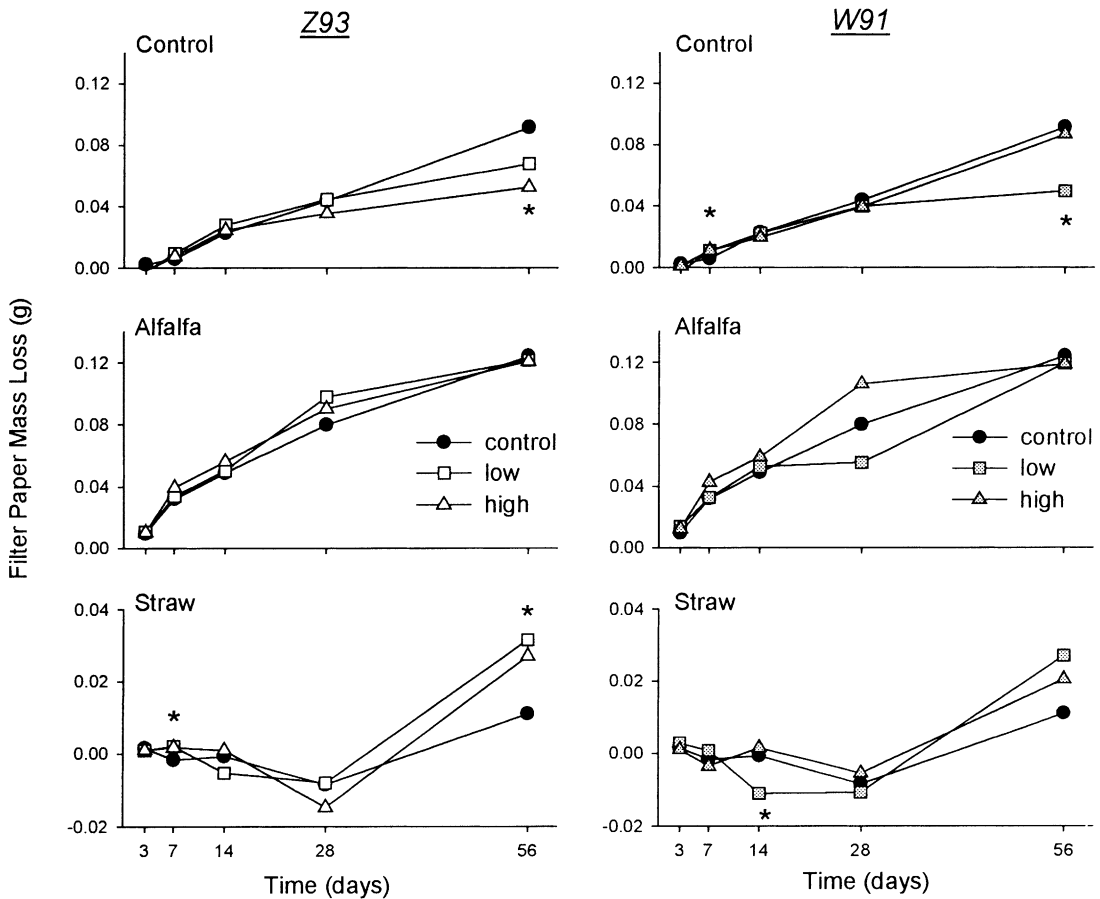


Fig. 4. Cellulase enzyme activity of soils treated with Z93 and W91 at two concentrations (mean ± S.E.). Symbols indicate significant chemical effects for each date at $P < 0.05$ (*).

soil were very similar (data not presented for reasons of space). There were clear differences in the dose-response relationships of the two biostimulants. Z93 stimulated soil respiration consistently over a wide range of concentrations, and stimulated DHA at relatively low concentrations (0.5–500 nl/g soil). W91 stimulated soil respiration at higher concentrations (0.5–50 µl/g soil), but depressed respiration at the highest concentrations used. Both biostimulants appeared to inhibit DHA at the higher concentrations (0.5–50 µl/g soil). Clearly, both biostimulants can either stimulate or inhibit specific components of overall soil microbial activity, depending upon the concentrations used.

3.2. Eight-week soil incubations

Results of *F*-tests from an overall ANOVA table for measured variables are listed in Table 1, which includes the main effects of biostimulant treatment, soil amendment, and date, and their two- and three-way interactions. In addition, biostimulant concentrations were nested within each biostimulant treatment.

In general, rates of SIR increased during the first 2 weeks, and decreased to relatively low levels for the last two sample dates (days 28 and 56) (Fig. 2). The alfalfa-amended soil had a much greater SIR than either the control or the straw-amended soils, presumably because of the greater quantity of

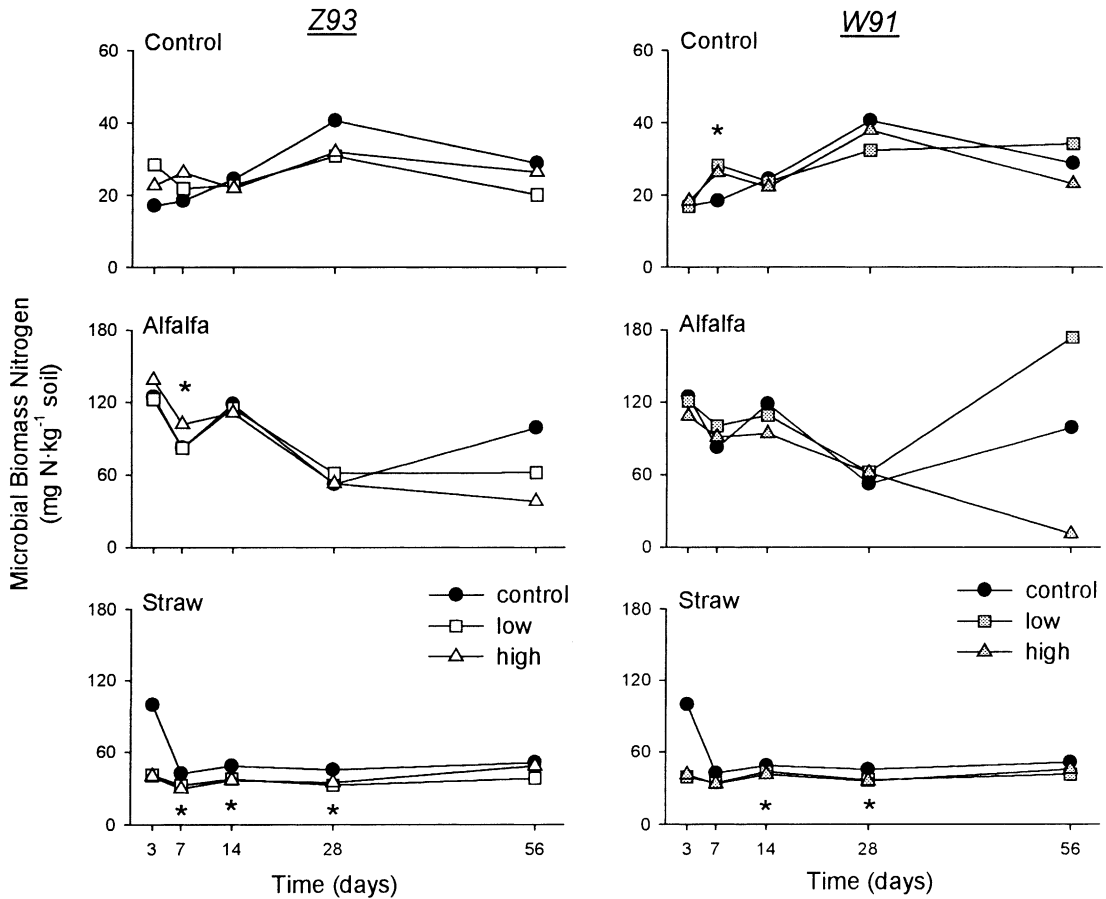


Fig. 5. Microbial biomass nitrogen concentration in soils treated with Z93 and W91 at two concentrations (mean ± S.E.). Symbols indicate significant chemical effects for each date at *P* < 0.05 (*).

readily-available carbon and nitrogen substrates for microbial growth and activity. Both Z93 and W91 treatments influenced SIR significantly, depending on the type of organic amendment and date. In the alfalfa- and straw-amended soils, both treatments generally stimulated SIR. However, in the control soil, Z93 resulted in lower rates of SIR. This suggests that the biostimulants may have enhanced the microbial utilization of the added organic substrates.

DHA was greatest in the first few days of incubation, and decreased steadily thereafter (Fig. 3). It is not entirely clear why DHA and SIR were not well-correlated, but it may be that DHA is primarily a measure of the active bacterial biomass, whereas, SIR is a better measure of the respiratory activity of both fungal and bacterial populations. As with

SIR, both compounds influenced DHA significantly ($P < 0.05$), depending on the type of organic amendment and date. W91 generally stimulated DHA in the control and alfalfa soils. Z93 also stimulated DHA in the control soil. Both compounds suppressed DHA on day 3 in the straw-amended soil. We expected fungal activity to be greatest in this amended soil, since it was treated with a high C:N ratio material. In this soil, the biostimulants may have inhibited bacterial activity and stimulated fungal activity in the early days of the incubation, thereby leading to lower DHA while increasing overall respiration rates.

Cellulase activity was measured using the rate of mass loss of cellulose filter papers placed in the soil containers as an index. Filter paper decomposition proceeded at similar rates in both the control and

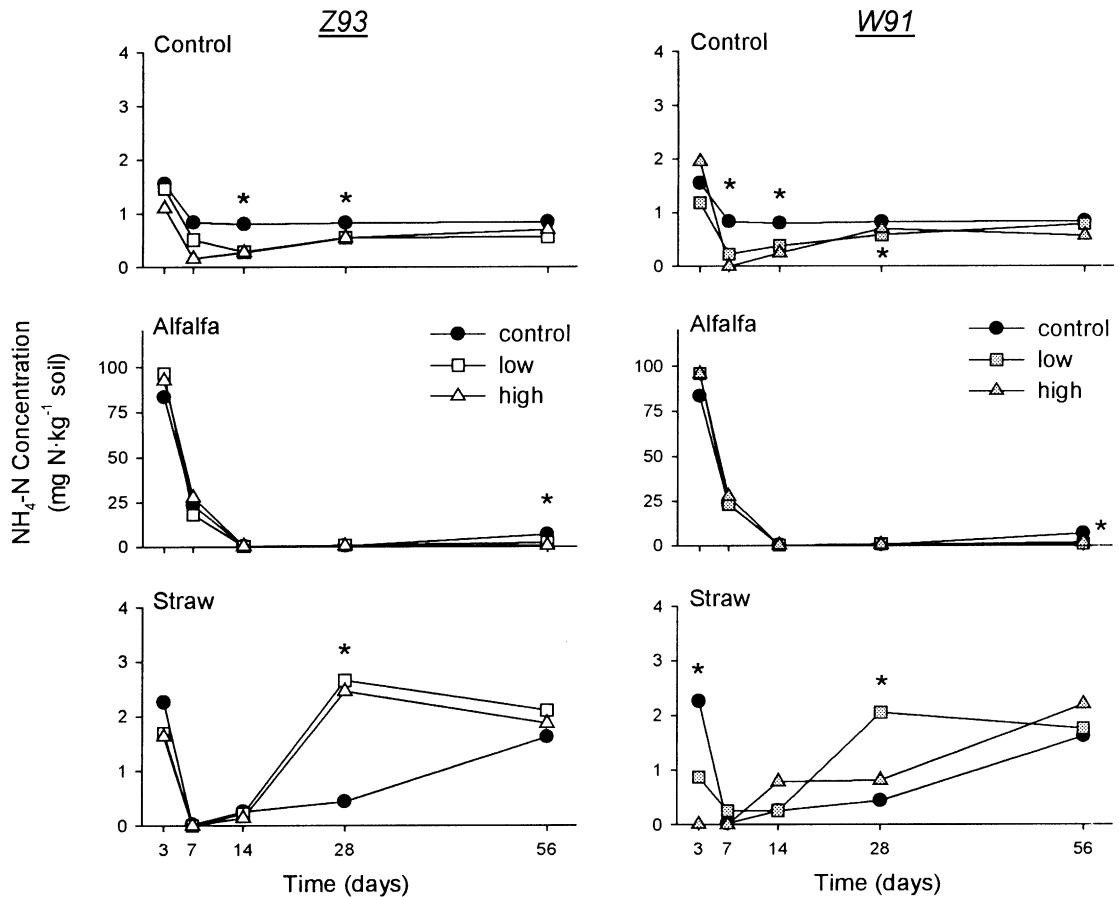


Fig. 6. Ammonium-nitrogen concentration in soils treated with Z93 and W91 at two concentrations (mean \pm S.E.). Symbols indicate significant chemical effects for each date at $P < 0.05$ (*).

alfalfa-amended soils, but was much slower in the straw-amended soil (Fig. 4). There were few differences between the biostimulant-treated soils and the controls, except for the last date in the control soil, where both Z93 and W91 decreased mass loss (i.e. there was less organic matter decomposition), and in the straw-amended soil, where Z93 increased mass loss (with greater decomposition) ($P < 0.05$). Cellulolytic activity in the soil is derived primarily from fungal activity. Therefore, these results suggest that the biostimulants can either stimulate or inhibit fungal activity, depending on the C:N quality of the available organic substrates.

Levels of microbial biomass nitrogen remained relatively constant during the soil incubations. In the first week in both the control and alfalfa-amended soils,

both Z93 and W91 produced significant increases in microbial biomass nitrogen compared to those in the controls (Fig. 5). However, in the straw-amended soil, both compounds led to consistently lower amounts of microbial biomass nitrogen than in the controls. Again, this suggests differential effects of the compounds, depending upon the quality of the organic materials in the soil. It is possible that under conditions of low nitrogen availability, such as in the straw-amended soil, the two biostimulants may have inhibited the growth of at least some components of the overall microbial biomass.

The alfalfa leaf amendment contributed large amounts of ammonium to the soil (Fig. 6), both through leaching from the added nitrogen-rich material and through subsequent rapid decomposition and

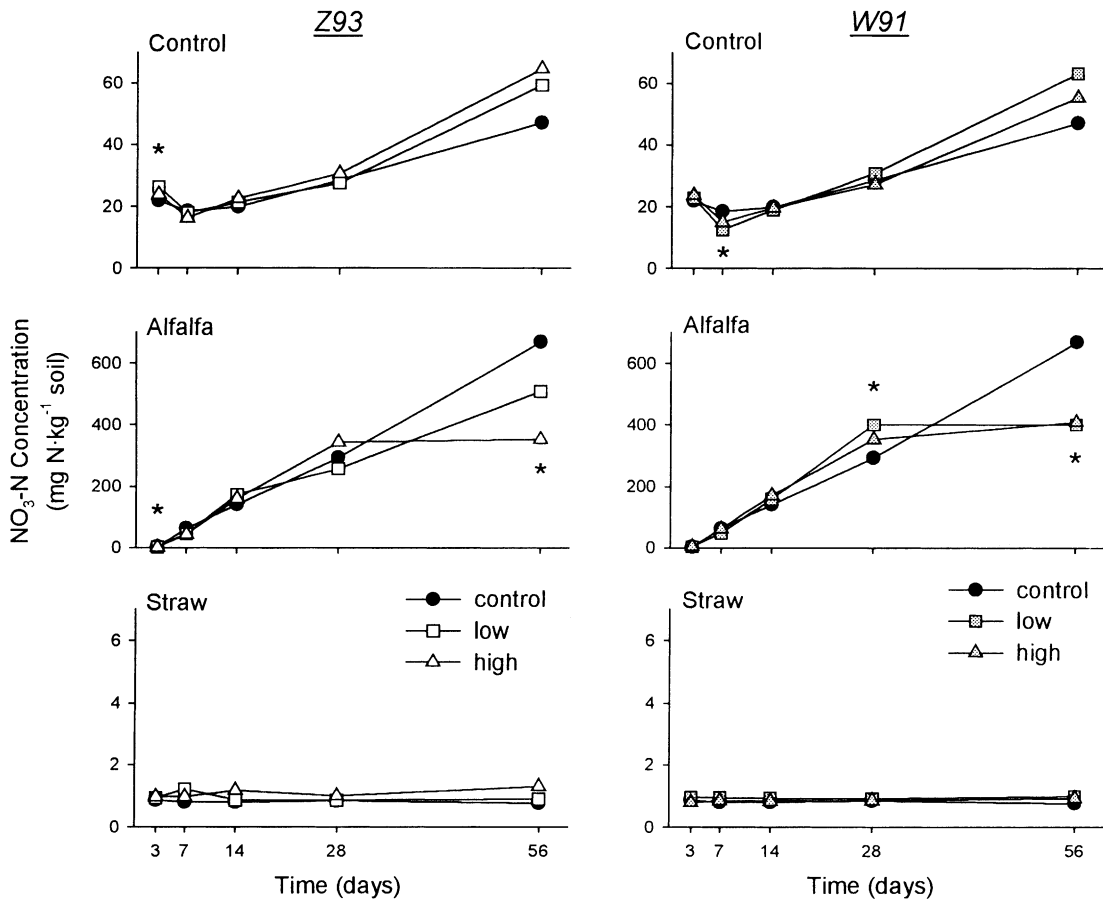


Fig. 7. Nitrate-nitrogen concentration in soils treated with Z93 and W91 at two concentrations (mean \pm S.E.). Symbols indicate significant chemical effects for each date at $P < 0.05$ (*).

mineralization (Subler et al., 1995). Initial (day 3) ammonium concentrations were greater in the alfalfa-amended soils treated with W91, than in the untreated controls, suggesting that W91 stimulated the early mineralization of nitrogen from the alfalfa leaf material. Both Z93 and W91 also increased ammonium concentrations relative to that in the controls on day 28 in the straw-amended soil. In the unamended control soils, both biostimulants reduced ammonium concentrations, suggesting either retarded mineralization, enhanced nitrification, or microbial uptake of ammonium.

Soil nitrate concentrations increased steadily during the incubations of the control and alfalfa-amended soils, accumulating to extremely high levels in the

alfalfa soil (Fig. 7). Conversely, nitrate concentrations were consistently low in the straw-amended soil throughout the incubation period, presumably due to very strong microbial immobilization of mineral nitrogen. The greatest influence of the biostimulant treatments was in the alfalfa-amended soil on day 56, when both compounds led to substantially lower concentrations of nitrate than that in the controls (a difference of >200 ppm $\text{NO}_3\text{-N}$). This may have resulted from decreased nitrogen mineralization, or increased microbial uptake of nitrogen. It is also possible that the biostimulants stimulated denitrification in these soils.

It is obvious that soluble organic materials may serve as a readily mineralizable source of nitrogen. Often, concentrations of soluble organic nitrogen in

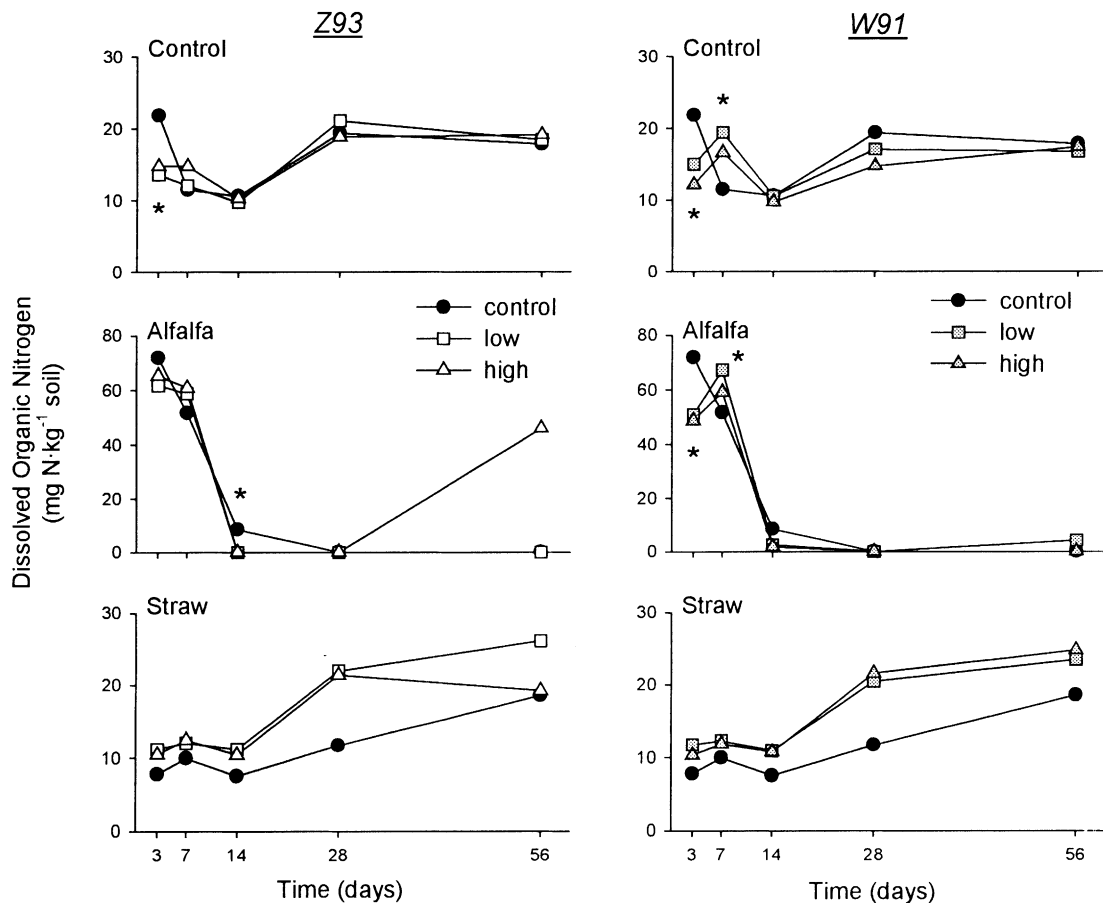


Fig. 8. DON concentration in soils treated with Z93 and W91 at two concentrations (mean \pm S.E.). Symbols indicate significant chemical effects for each date at $P < 0.05$ (*).

soils are large relative to those of inorganic nitrogen. In the control and alfalfa-amended soils, the biostimulant treatments tended to reduce DON on day 3, and increase DON on day 7, relative to that in the controls (Fig. 8). Apparently, the biostimulants delayed the development of peak concentrations of DON. In the straw-amended soil, however, both compounds led to consistently greater concentrations of DON than in controls. This is exactly opposite to the response of microbial biomass nitrogen. By inference, inhibition of microbial growth led to an accumulation of dissolved organic substances in the soil.

4. Conclusions

The results of these preliminary investigations into these two commercial biostimulants on nutrient availability indicate that small amounts of both Z93 and W91 can inhibit as well as stimulate soil microbial activities, depending on the concentration of the application, the quality of organic materials in the soil, and time. These changes are associated with alterations in soil nitrogen dynamics, which were apparent up to 56 days after soil treatment. The results suggest that the two biostimulants may have hindered the breakdown and mineralization of soil organic materials, perhaps by selectively inhibiting or stimulating particular components of the microbial community (i.e. fungal versus bacterial), leading to lasting (8 week) effects on soil nitrogen availability. Many of the observed changes in soil microbial biomass and activity in our study were consistent with increased fungal activity or reduced bacterial activity. Indeed, we noticed in many of the incubations that treatment with both biostimulants, and particularly Z93, led to the rapid development of fungal hyphae on the surface of the incubating soil. The biostimulants may inhibit or promote alterations in the microbial community in other ways, such as by supplying micronutrients or other growth factors, augmenting enzymatic activity in the soil, or by chemically inhibiting specific microbial populations. Additional research is needed to determine if the effects observed in our small-scale soil incubations can be extrapolated to explain those that have been obtained consistently in more realistic field systems that include growing plants. Nevertheless, this laboratory work provides strong evidence that small

amounts of agricultural biostimulants can affect soil microbial activity and nitrogen dynamics significantly even when applied to soil at the extremely low recommended as concentrations as biostimulants to enhance crop growth.

Acknowledgements

Thanks to David Williams and Margaret Huelsman for their help in the laboratory. This research was funded by a grant from AgSpectrum Company to The Ohio State University.

References

- Ag Spectrum Co., 1996. Proof Book, 1995–1996. Ag Spectrum Co., DeWitt, Iowa.
- AgroPlus, Inc., 1991. Grozyme as a yield enhancer and soil nitrogen booster for agricultural use: a summary of field trials and replicated studies 1979–1990, Vol. 1. Unpublished report, AgroPlus Inc., Hawkins, Texas.
- AgroPlus, Inc., 1992. Grozyme as a yield enhancer and soil nitrogen booster for agricultural use: a summary of field trials and replicated studies 1991, Vol. 2. Unpublished report, AgroPlus Inc., Hawkins, Texas.
- Anderson, J.P.E., 1982. Soil respiration. In: Page, A.L. (Ed.), *Methods of Soil Analysis, Part 2, Chemical and microbiological properties*, Agronomy Monograph No. 9, 2nd Edition. ASA-SSSA, Madison, WI.
- Brookes, P.D., Stark, J.M., McIneer, B.B., Preston, T., 1989. Diffusion method to prepare soil extracts for automated nitrogen-15 analysis. *J. Soil Soc. Sci. Am.* 53, 1707–1711.
- Cabrera, M.L., Beare, M.H., 1993. Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. *J. Soil Sci. Soc. Am.* 57, 1007–1012.
- Casida Jr., L.E., 1977. Microbial metabolic activity as measured by dehydrogenase determinations. *Appl. Environ. Microbiol.* 34, 630–636.
- Chen, Shu-Kang, Edwards, C.A., Subler, S., 2002. The influence of two agricultural biostimulants on nitrogen transformations, microbial activity, and plant growth in soil microcosms. *Soil Biol. Biochem.*, in press.
- Conover, W.J., Iman, R.L., 1981. Rank transformation as a bridge between parametric and nonparametric statistics. *Am. Statistician* 35, 124–133.
- Miller, R.H., 1990. Soil microbiological inputs for sustainable agricultural systems. In: Edwards, C.A., Lal, R., Madden, P., Miller, R.H., House, G. (Eds.), *Sustainable Agricultural Systems*. Soil Water Conservation Society, pp. 614–623.
- Subler, S., Blair, J.M., Edwards, C.A., 1995. Using anion-exchange membranes to measure soil nitrate availability and net nitrification. *Soil Biol. Biochem.* 27, 911–917.