SHORT COMMUNICATION

E. E. Kara · M. Arli · V. Uygur

Effects of the herbicide Topogard on soil respiration, nitrification, and denitrification in potato-cultivated soils differing in pH

Received: 9 December 2002 / Accepted: 5 January 2004 / Published online: 26 February 2004 © Springer-Verlag 2004

Abstract We investigated the effects of Topogard 50 WP (3 kg ha⁻¹) on soil respiration, mineral N content, and number of denitrifying and total bacteria in four coarsetextured volcanic soils for 91 days. Topogard application decreased CO₂ evolution in acid soils (Tepedibi and Karaçakıl) whereas soil respiration was initially increased in neutral and alkaline soils (Kaşbaşı and Bağlar). The herbicide application significantly stimulated ammonification in Kaşbaşı and Bağlar soils, while Tepedibi and Karaçakıl soils showed significantly lower NH4⁺-N contents than the control. The treatment inhibited the activity of nitrifying microorganisms and, thus it decreased the NO₃⁻-N content in Tepedibi, Karaçakıl, and Kaşbaşı soils, whereas the NO₃⁻-N content was increased in Bağlar soil. The NO₂⁻-N content of soils was not affected by the treatment. The activity of denitrifying bacteria was stimulated by the addition of herbicide in all soils, whereas the total number of bacteria was not influenced. It may be concluded that the effects of Topogard on the microbiological characteristics of coarse-textured soils are likely to be dependent on soil pH.

Keywords Topogard 50 WP · Carbon dioxide production · Mineralization · Bacteria number · pH

Introduction

Chemical control of weeds in arable lands is a common agricultural practice used throughout the world to maximize and enhance the quality of agricultural products. However, applied herbicides can affect soil microorgan-

M. Arli · V. Uygur (⊠) Agricultural Faculty, Soil Science Department, Mustafa Kemal University, 31040 Antakya, Hatay, Turkey Tel.: +326-245-5836/1355 Fax: +326-245-5832 isms and may disturb N and C cycles, which are very critical for soil quality. Herbicide applications may increase/decrease or inhibit the microbiological activity and its related components in the soil environment for different periods of time depending on the soil characteristics and active ingredients of the herbicides (Cernakova et al. 1991; El-Shanshoury et al. 1997; Gigliotti et al. 1998; Tu 1996). Singh and Wright (1999, 2002) reported that terbutryn/terbuthylazine, trietazine/ simazine prometryn and bentazone, all triazine derivatives, negatively affected the growth of rhizobia.

Munch et al. (1989) reported that the activity of NO_2^{-} oxidizing bacteria was inhibited by terbuthylazine, whereas the activity of NH_4^+ -oxidizing and denitrifier bacteria was stimulated by the herbicide. Ferrero and Maggiore (1994) reported that alachlor+terbuthylazine application to a cattle slurry given sandy soil under maize cultivation reduced NO_3^- leaching probably due to the inhibition of nitrification.

Salminen et al. (1997) found that exposure of a coniferous soil in microcosms to terbuthylazine increased N mineralization rates, whereas El-Shanshoury et al. (1997) reported that terbutryne stimulated the growth of asymbiotic N-fixing *Azotobacter chroococcum* strains.

The solubility and ionic forms of triazines and their derivates are largely affected by soil pH, substitution at the R1 position (Yaron et al. 1996) and adsorption ability of soil components (Walker 1991). As a result, their effects in the soil environment can change depending on soil characteristics such as pH and organic matter content.

Even though Topogard 50 usage is not suggested for light-textured soils, this herbicide, with a market share of >40% in the last decade, has been used for controlling weeds in light-textured potato-cultivated soils of Nigde, Turkey. Therefore, the aim of this study was to investigate the effects of Topogard 50 WP, a composite of heterocyclic N derivates (34% terbutryne+%15 terbuthy-lazine+1% related compounds), at a typical field application rate (3 kg ha⁻¹) on soil microbiological activity, mineral N content (NH₄⁺-N, NO₂⁻-N and NO₃⁻-N), and

E. E. Kara

Faculty of Engineering, Environmental Engineering Department, Niğde University, Niğde, Turkey

total and denitrifier bacteria in four potato-cultivated volcanic soils differing in pH.

Materials and Methods

Soils

Four different soils under a potato monoculture were sampled from villages near Niğde (37°25′, 38°58′N; 33°10′, 35°25′E). The soils were surface (0–20 cm) sampled from the Himmetli Tepedibi site, the Kilederesi Karaçakıl site, the Himmetli Kaşbaşı site, and the Ağcaşar Bağlar site, and all were formed on the volcanic parent materials under semi-arid continental climate conditions. After sampling, soils were air-dried and sieved (<2 mm). The pH (1:2.5 soil: water mixture), total dissolved salts (US Salinity Laboratory Staff 1954), organic C wet combustion, total N, available P (Olsen et al. 1954), and CaCO₃ equivalent of the soils were measured as reported by Sparks (1996); the soil particle size (Bouyoucus 1951) and field capacity were also determined (Klute 1986). Chemical and physical properties of soils are reported in Table 1.

Incubation experiments

Topogard 50 WP (34% terbutryne+%15 terbuthylazine+%1 related compounds) was thoroughly mixed with soil [equivalent to 100 g oven-dry soil (ODS)] at a rate of 3 kg ha⁻¹ (application rate to field soil: 2–3 kg ha⁻¹). Treatments were repeated 3 times; control treatments included soils with no herbicide addition. Then soils were incubated in tightly closed plastic containers at 25±2 C for 91 days. The moisture content of soil samples was kept constant at 60% of field capacity during the course of the incubation. Mineral N forms (NH₄⁺, NO₃⁻, and NO₂⁻), the number of total bacteria and denitrifier bacteria were determined after 1, 7, 14, 21, 28, 35, 42, 56, 70, and 84 days, whereas soil CO₂ production was determined daily in the first week and then at weekly intervals up until day 91.

Chemical and microbiological analysis

Mineral N forms were extracted by shaking soils (equivalent to 10 g ODS samples) with 50 ml of 1% KAl(SO₄)₂ solution for 10 min; a clear supernatant was obtained after filtering. Then, NH_4^{+} -N, NO_2^{-} -N and NO_3^{-} -N contents of filtrates were colorimetrically determined by the Na-nitroprussic (Fachgruppe Wasserchemie in der Gesellchaft Deutscher Chemicer 1983), the naphthylamine (Nicholas and Nasan 1957), and the Na-salicylate (Schlichting and Blume 1966) methods, respectively.

 CO_2 produced was trapped in 20 ml of 0.1 M Ba(OH)₂ solution which was then titrated with the 0.045 M HCl solution (Isermeyer 1952).

Total bacteria and denitrifiers were counted in Tombinson and Hochstein host media as described by Ottow (1984).

Statistical analysis

ANOVAs and paired comparison of means by Duncan's test were performed by using SPSS.

Results and discussion

The effects of Topogard application on soil CO₂ production are given in Fig. 1A, B. The soils may be divided into two groups according to the effect of the herbicide. CO₂ evolution was significantly increased (P < 0.05 or P < 0.01) by herbicide application in the Tepedibi and Kaşbaşı soils after 1, 2 and 3 weeks, respectively. Then, higher rates of CO₂ evolution were recorded for the control treatments of both soils. These results suggest that soil microorganisms consumed the added herbicide as C and/or N sources under favourable water and temperature conditions, as stated by Haktanır and Arcak (1998). It may be also possible that soil microorganisms consumed readily available C at the beginning of the incubation (Kara 1999).

In contrast, these results were not observed in the Karaçakıl and Bağlar soils, which are both acidic (pH 5.5 and 4.5, respectively). Since the solubility of both terbutryne and terbuthylazine, which are triazines, increases with decreasing pH (Yaron et al. 1996) it may be possible that this herbicide has a more toxic effect in Karaçakıl and Bağlar soils than in Tepedibi and Kaşbaşı soils. However, their different behaviour may also depend on differences in the microfloral composition of these soils (Alexander 1977).

The effects of Topogard application on the amount of exchangeable NH_4^+ are shown in Fig. 1C, D. The contents of exchangeable NH_4^+ in Tepedibi and Kaşbaşı soils did not show a significant difference between treated and untreated soils (Fig. 1C, D).

The exchangeable NH_4^+ contents of untreated Karaçakıl and Bağlar soils were always higher than those of the respective herbicide-treated soils, but the differences were never statistically significant (Fig. 1C, D). As has been hypothesized for the CO₂ evolution in this study, the soils may differ in the composition of their microflora and this may have an effect on the ammonification process in them. Greater NH_4^+ concentrations were observed in acidic Karaçakıl and Bağlar soils than in the slightly alkaline Tepedibi and Kaşbaşı soils. These differences could be due to the reduction in nitrification at pH values

Table 1 Chemical and physicalproperties of the soils

Soil property	Tepedibi	Karaçakıl	Kaşbaşı	Bağlar
Texture	Sand	Loam	Loam	Sand
pH (in saturation paste)	7.20	5.50	7.50	4.50
Organic matter (%)	1.35	1.22	1.00	1.22
Field capacity (%) Total dissolved salts (%) (in saturation paste)	36.6	40.3	40.2	37.4
Total N (%) Available P (P_2O_5 kg ha ⁻¹)	0.087 64.1	0.078 64.1	0.076 13.7	0.082 46

Fig. 1 Effect of herbicide application on CO_2 evolution (A, B), NH⁺ content (C, D), NO₃⁻ content (E, F), and number of denitrifying bacteria (G, H). *ODS* Oven-dry soil



<6 since nitrification but not ammonification is affected by acid pH values.

Nitrification in the Tepedibi and Kaşbaşı soils was significantly inhibited at the beginning of the incubation period, but was then stimulated by Topogard (Fig. 1E, F). This may have been related to the persistence of the herbicide, that is nitrification may have been stimulated when the herbicide was degraded. Decomposition products of the herbicide appear after 7 days and the half-life of terbuthylazine changes from 14 to 22 days depending on the availability of N (Leita et al. 1996). Munch et al. (1989) reported an increase in the activity of NH₃-

oxidizing and nitrifying bacteria at rates of 1.5, 150, and 1,500 mg terbuthylazine kg⁻¹. In contrast, atrazine and simazine (triazine derivates) applied at rates of 10, 50 and 100 mg 1^{-1} reduced the NO₃⁻ content of soils (Somda et al. 1990).

Karaçakıl and Bağlar soils showed similar behaviour because in both the herbicide reduced the nitrification rate during the incubation, but the difference was statistically significant only for the Karaçakıl soil up to 3 weeks of incubation (Fig. 1E, F). Amantaev et al. (1979) reported that simazine applied at field rates increased nitrification in acid soils, and probably this depends on the fact that simazine is less soluble than other triazine derivates (Hassall 1990). Generally, the NO_3^- concentration in the herbicide-treated soils did not decrease during the incubation period due to the absence of leaching and plant uptake.

The effect of the herbicide on the NO_2^- concentration of soils, which ranged from 0.00 to 0.119 mg kg⁻¹, was found to be negligible (data not shown). It is well known that NO_2^- is immediately oxidized to NO_3^- .

The presence of the herbicide did not change significantly the total number of bacteria in soils during the incubation (data not shown). The number of bacteria was 1.1×10^5 for all soils and increased up to 1.4×10^5 after the first (in Karaçakıl soil with and without treatment) and the second week (the other soils) and finally decreased to 1.1×10^5 after the sixth week of the incubation. No significant differences among different soils were observed.

The herbicide application significantly (P < 0.01) increased the number of denitrifying bacteria in soils, but the initial effect was not significant for Tepedibi and Karaçakıl soils (Fig. 1G, H). The number of denitrifying bacteria depends on NO₃⁻-N content and C availability, soil pH, water content and temperature of soil (Paul and Clark 1996). Since water content and temperature were the same for all soils the most critical factor limiting denitrifying bacteria in our experimental conditions were NO₃⁻-N content, C availability and pH.

The initial increases in the NO₃⁻-N content of treated and untreated soils were accompanied by an increase in the number of denitrifying bacteria (Fig. 1G, H). A NO₃⁻ concentration exceeding 20 mg l⁻¹ in soil solution has been found to stimulate the denitrifying activity of soil bacteria if available C is present (Paul and Clark 1996). When the NO₃⁻-N content along with the C content decreased, the number of denitrifying bacteria declined rapidly; such a decrease occurred after 4 weeks in Tepedibi, Karaçakıl and Bağlar soils and after 5 weeks in Karaçakıl soil. Although the NO₃⁻-N content remained fairly high the number of denitrifying bacteria decreased after the fourth or fifth week and this was related to the depletion of available C sources and decomposition and/ or disappearance of the herbicide in this period (half-life 14–22 days).

The average numbers of denitrifying bacteria of herbicide-applied soils were inversely related to the soil pH, so that their relative order was Kaşbaşı> Tepedibi>Karaçakıl>Bağlar. The optimum activity of denitrifying bacteria occurs at pH 6–8 (Paul and Clark 1996).

After the first week, the herbicide application statistically increased the percentage of total bacteria present as denitrifying bacteria in Tepedibi, Karaçakıl, Kaşbaşı soils, while such an increase occurred in Bağlar soil after 3 weeks. This increase ranged from 0.61 to 9.5% and was smaller than that (50–60%) reported by Kara (1999) in fine-textured soils, with a higher organic matter content. A lower organic C content and more acidic values of our soils than those used by Kara (1999) may explain the difference in the percentage of denitrifying bacteria in the two studies.

In conclusion, soil pH might be one of the most important properties regulating the effects of the herbicide Topogard on microbial activities, including N reactions, in coarse-textured soils. Since this herbicide stimulated the denitrifying activity, the possible evolution of nitrous oxides from such treated soils must be considered. On the other hand, an increase in the nitrification rate in coarse-textured soils can further reduce the efficiency of fertilizers. Therefore, before using any herbicides the benefits and potential hazards of their use must be carefully considered.

References

- Alexander M (1977) Introduction to soil microbiology, 2nd edn. Wiley, New York
- Amantaev E, Ilyaletdinov A, Kudshev T (1979) Agrobiologia. Adv Agron 31:47–55
- Bouyoucus GJ (1951) A recalibration of the hydrometer method for making a mechanical analysis of soils. Agron J 43:434–438
- Cernakova M, Kurucova M, Fuchova D (1991) Effect of the herbicide bentanex on soil microorganisms and their activity. Folia Microbiol 36:561–566
- El-Shanshoury RA, Mohamed YA, Zeid AMA (1997) Growth aspects of selected *Azotobacter* and streptomyces species in response to the herbicide terbutryn. Acta Microbiol Pol 46:285– 295
- Fachgruppe Wasserchemie in der Gesellchaft Deutscher Chemicer (eds) (1983) Deutsche Einheitsuerfahren zur Abwasser und Schlammuntersuchungen. Chemie-Verlag, Weinheim
- Ferrero A, Maggiore T (1994) Leaching of slurries and herbicides in subsurface water under field conditions. In: Borin M, Sattin M (eds) Proceedings of the Third Congress of the European Society for Agronomy, Padova University, Abano-Padova, Italy. pp 794–795
- Gigliotti C, Allievi L, Salardi C, Ferrari F, Farini A (1998) Microbial ecotoxicity and persistence of the herbicide bensulfuron methyl in soil. J Environ Sci Health B 33:381–398
- Haktanır K, Arcak S (1998) Environmental pollution (in Turkish). Ankara University Agriculture Faculty publication no. 1503, text book no. 457. Ankara University Agriculture Faculty, Ankara
- Hassal KA (1990) The biochemistry and uses of pesticides, 2nd edn. Macmillan, London
- Isermeyer H (1952) Eine einfache methode zur bestimmung der bodenatmung und der karbonate im boden. Z Pflanzenernaehr Bodenkd 5:56–60
- Kara EE (1999) Gelemen tarım işletmesindeki toprak serilerinde, inkübasyon süresine bağlı olarak bazı mikrobiyolojik özelliklerde meydana gelen değişmeler. Turk J Agric For [Supplement 2] 23:459–466
- Klute A (1986) Methods of soil analysis: physical and mineralogical methods, part 1, 2nd edn. ASA, SSSA, Madison, Wis.
- Leita L, Ceccon P, Marucchini C, Mondini C (1996) Behavior of metolachlor and terbuthylazine in cultivated field lysimeters. Z Pflanzenernaehr Bodenkd 159:177–182
- Munch JC, Gloth B, Henneberger C (1989) The effect of terbuthylazine on soil microorganisms of the nitrogen cycle. Einfluss Eines Terbutylazine Praparates Bodenmikroorganizmen des Stickstoff Kreislaufs. Mitt Deutsch Bodenkd Ges 59(1):603– 606
- Nicholas DS, Nasan A (1957) Determination of nitrate and nitrite. In: Colowick SP, Kaplan NO (eds) Methods in enzymology. Academic Press, New York, pp 981–984

- Olsen SR, Cole CV, Watanabe FS, Dean LA (1954) Estimation of available phosphorous in soil by extraction with sodium bicarbonate. USDA circular 939. USAD, Washington, D.C.
- Ottow JCG (1984) Bodenmikrobiyologisch-biochemisches Practicum 1–2
- Paul EA, Clark FE (1996) Soil microbiology and biochemistry. Academic Press, San Diego, Calif.
- Salminen J, Seyala H, Haimi J (1997) Regulation of decomposer community structure and decomposition processes in herbicide stressed humus soil. Appl Soil Ecol 6:265–274
- Schlichting E, Blume H (1966) Bodenkundliches Prakticum. Springer, Berlin Heidelberg New York
- Singh G, Wright D (1999) Effects of herbicides on nodulation, symbiotic nitrogen fixation, growth and yield of pea (*Pisum sativum*). J Agric Sci 133:21–30
- Singh G, Wright D (2002) In vitro studies on the effects of herbicides on the growth of rhizobia. Lett Appl Microbiol 35:12–16

- Somda SC, Mills HA, Phatak SL (1990) Nitrapyrin, terrazole, atrazine and simazine influence on nitrification and corn growth. J Plant Nutr 13:1179–1193
- Sparks DL (1996) Methods of soil analysis. Part 3. Chemical methods. ASA, SSSA, Madison, Wis.
- Tu CM (1996) Effect of selected herbicides on activities of microorganisms in soils. J Environ Sci Health B 31:1201–1214
- US Salinity Laboratory Staff (1954) Diagnosis and improvement of saline and alkaline soils. USDA circular no. 60. USDA, Washington, D.C.
- Walker A (1991) Influence of soil and weather factors on the persistence of soil applied herbicides. Appl Plant Sci 5:94–98
- Yaron B, Calvet R, Prost R (1996) Soil pollution. Processes and dynamics. Springer, Berlin Heidleberg New York