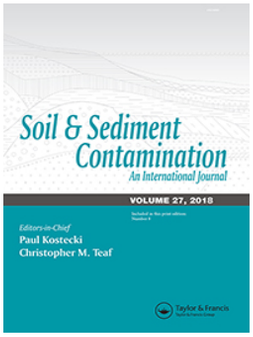

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Degradation and Detoxification of Nicosulfuron by a *Pseudomonas* Strain Isolated from a Contaminated Cornfield Soil

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ABSTRACT

The dissipation and detoxification of nicosulfuron (NS) by *Pseudomonas aeruginosa* B9 isolated from a cornfield soil was investigated. The fastest decline of NS occurred at 40 µg ml^{-1} in liquid media with 0.25% glucose plus 0.05% yeast extract (DT_{50} = 4 days) with a notable pH reduction (pH < 5). Bioassay tests showed considerable phytotoxicity of NS for Cress (*Lepidium sativum* L.) with 50% shoot growth inhibition (SGI) at 40 µg ml^{-1}. The dissipation of NS (40 µg ml^{-1}) by the B9 isolate reduced the SGI significantly (SGI: up to 45 ± 3%) compared to the non-inoculated media (SGI: up to 58 ± 4%). In soils with the B9 isolate, NS dissipation, especially at 0.3 µg g^{-1}, was faster with a more significant SGI reduction (k = 0.08 ± 0.00 day^{-1}; SGI = 2 ± 1%) compared to non-inoculated samples (k = 0.03 ± 0.00 day^{-1}; SGI = 8 ± 1%). NS initially inhibited soil respiration, microbial biomass carbon, and dehydrogenase activity. The effect was however transient, and these parameters recovered within 10 days, especially in the presence of the isolate. Overall, this study proves *Pseudomonas aeruginosa* B9 as a suitable candidate for bioremediation of NS in contaminated sites.

KEYWORDS

Nicosulfuron; pseudomonas aeruginosa; *bioassay*; *bioremediation*; *microbial activity*

Introduction

Nicosulfuron (NS) (2-[(4,6-dimethoxypyrimidin-2-ylcarbamoyl) sulfamoyl]-N,N-dimethylnicotinamide) is a post-emergence sulfonylurea herbicide widely used to suppress various grasses and broadleaf weed species in cornfields. The herbicide (SC 4%) is applied at a rate of 2 l ha^{-1} at two- to four-leaf stage of corn (Baghestani *et al.*, 2007). NS is known to be highly mobile in soil, and several studies have addressed soil and water contamination by this herbicide (Battaglin *et al.*, 2000; Regitano and Koskinen, 2008). In addition, adverse effects of NS and other similar sulfonylurea herbicides on non-target organisms such as algae and phytoplankton have been reported (Sabater *et al.*, 2002; Seguin *et al.*, 2001; Yue *et al.*, 2007). As a result, expanding bioremediation strategies for elimination of NS residues from contaminated sites are inevitable.

Interactions between pesticides and soil microorganisms can lead to microbial degradation of these compounds that is one of the most dominant pathways involved in their decline

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and detoxification (Sørensen *et al.*, 2008). Although sulfonylurea herbicides may undergo several abiotic attenuation processes in soil such as photolysis (Herrmann *et al.*, 1985; Ter Halle *et al.*, 2010) and chemical hydrolysis (Perreau *et al.*, 2007; Sarmah and Sabadie, 2002), biodegradation is considered as the main mechanism involved in their dissipation (Boschin *et al.*, 2003; Kang *et al.*, 2012; Sondhia *et al.*, 2013). Therefore, bioremediation of these herbicides by using isolated microbial cultures has received extensive attention. So far, only a few pieces of research have addressed the biodegradation of NS by isolated microorganisms from soils. Based on these studies, the isolated microorganisms were capable of degrading NS mainly through cleavage of the C-N bond or sulfonylurea bridge (Feng *et al.*, 2017; Lu *et al.*, 2012; Wang *et al.*, 2016; Yang *et al.*, 2008; Zhang *et al.*, 2012; Zhao *et al.*, 2015; Zhou *et al.*, 2017). However, whether the degradation of NS actually detoxifies the herbicide and reduces its phytotoxicity for a test plant, for example, by producing less toxic transformation products, has not been elucidated in previous research. We hypothesize that soils with histories of NS application carry indigenous degrading microorganisms that can be isolated and used for both degradation and detoxification of the herbicide.

Another aspect of pesticide-microbe interactions is the way these chemicals affect soil microbial activities. Microorganisms play a key role in soil biogeochemical cycles, and their action is critical for maintaining and boosting the soil quality (Topp, 2003). Therefore, putative effects of pesticides on soil microbial functions are of great concern to many researchers (Carpy *et al.*, 2000; Ritz and Rull, 2008). It has been shown that exposure of soils to pesticides can affect microbial activity parameters such as soil respiration (SR), microbial biomass carbon (MBC) (Dutta *et al.*, 2010; Radivojević *et al.*, 2012), and dehydrogenase activity (DA) (Stepniewska *et al.*, 2007). These effects can be stimulatory or inhibitory (Imfeld and Vuilleumier, 2012), and their extent may vary depending on the soil and pesticide properties (Dungan *et al.*, 2003; Gevao *et al.*, 2000) and the initial concentration of the applied pesticide (Dungan *et al.*, 2003; Gomez *et al.*, 2009). Although NS is known to inhibit the growth of some special bacterial populations such as the actinomycetes (Šantrić *et al.*, 2016) and *Clostridium* sp. (Filimon *et al.*, 2015), research addressing the effects of this herbicide on soil microbial activity parameters is scarce. According to one study, exposure to NS reduced SR, MBC, and DA significantly, and the effects were transient and dependent on the concentration of the herbicide (Radivojević *et al.*, 2012). NS has a low soil sorption coefficient (K_{ow}) and, therefore, is considered a potentially mobile and bioavailable herbicide in soil (Table S1) (EPA, 1990; FAO, 2011). Therefore, we hypothesize that the herbicide may initially affect soil microbial activities significantly even at field-relevant concentrations.

To explore the hypotheses raised above, we conducted this research and aimed to i) isolate an NS-degrading bacterium from a cornfield soil, ii) investigate the ability of the isolate to dissipate and detoxify NS in liquid media and soil, and iii) assess the possible effects of NS on soil microbial activity parameters including SR, MBC, and DA.

Material and methods

Chemicals

NS standard (97.7%), deionized water, Orthophosphoric acid (H_3PO_4), acetonitrile (ACN) and ethyl acetate (EtOAc) (HPLC grade, purity: > 99.9%), and anhydrous magnesium sulfate (reagent grade: > 97%) were obtained from Sigma-Aldrich, Germany. Primary-

secondary amine (PSA) was supplied by Supelco, USA. Bacteriological agar was purchased from Liofilchem, Italy. All other chemicals and the salts used in the experiments were obtained from Merck, Germany. For the enrichment, degradation, and bioassay tests, a stock aqueous solution of filter-sterilized NS (0.2 μm, Hydrophilic PTFE, Millex®, USA) was prepared at 1 g l^{-1} in deionized water. For chromatographic analyses, NS was dissolved in ACN (1 g l^{-1}). All the stock solutions were kept at −20°C.

Soil sampling and characterization

The soils were sampled from a cornfield located in agriculture research farms of the University of Tehran (Longitude: 50° 57′ 22.72″ E, Latitude: 35° 48′ 22.64″ N, Altitude: 1294.38 m) in Karaj city. The field had been exposed to NS for several years. Using a flame-sterilized shovel, 10 samples (25 kg) were collected diagonally from the upper soil layer (0–15 cm), transferred to the laboratory, and kept at 4°C. Prior to experiments, samples were mixed, homogenized, and sieved (2 mm mesh). A portion of the prepared soil samples was used to measure physical and chemical properties including texture (Page *et al.*, 1982), organic matter (OM), pH (in water), maximum water holding capacity (MWHC), total nitrogen (N_{tot}), electrical conductivity (EC), and some major and trace elements (ISO standard procedures; http://www.iso.org). The soil physical and chemical properties are listed in Table 1.

Isolation, selection, and identification of NS-degrading microorganisms

The culture media used in the experiments included an enrichment (EM), a base (BM), and a potato dextrose agar (PDA) medium prepared according to Yang *et al.* (2008). The components of culture media are listed in Table S2 (SI section B).

Enrichment of NS-degrading microorganism was initiated in Erlenmeyer flasks containing EM + NS (500 μg ml^{-1}) inoculated with 1 g of the air-dried soil in triplicate. For controls,

Table 1. Physical and chemical properties of the experimental soil

Soil texture classification (FAO and USDA system)	Clay loam
Sand (%)	32.6 (2.1)*
Silt (%)	38.6 (1.7)
Clay (%)	28.8 (1.3)
pH (in water)	8.3 (0.5)
MWHC (%)	18.8 (0.6)
OM (%)	1.1 (0.1)
N_{tot} (%)	1.3 (0.2)
EC (ds m^{-1})	2.2 (0.3)
Ca (meq l^{-1})	16.2 (1.0)
Na (meq l^{-1})	4.5 (0.4)
K (meq l^{-1})	0.2 (0.1)
Mg (meq l^{-1})	7.2 (0.3)
P (μg g^{-1})	21.2 (1.2)
Fe (μg g^{-1})	3.4 (0.5)
Zn (μg g^{-1})	0.7 (0.3)
Cu (μg g^{-1})	1.2 (0.4)
Mn (μg g^{-1})	2.2 (0.8)

*Means of triplicated measurements with standard deviations in parentheses.
MWHC: maximum water holding capacity, **OM**: organic matter, **N_{tot}**: total nitrogen, **EC**: electrical conductivity

soils were sterilized (Berns *et al.*, 2008), and 1 g was added in triplicate to flasks containing EM + NS (500 μg ml^{-1}). Following 14 days of incubation at 30°C and 150 rpm, 5 ml of well-mixed grown cultures in each flask was transferred to fresh EM + NS (200 μg ml^{-1}) and incubated in the dark at 30°C and 150 rpm. After five rounds of enrichment within the same conditions, 0.1 ml tenfold dilution series of each liquid culture was spread on PDA + NS (200 μg ml^{-1}). The plates were incubated at 30°C for 48 h, and 10 morphologically distinct bacterial colonies were separated and purified. Colony characteristics of the isolates are presented in Table S3 (SI section C). For each isolate (B1-B10), an inoculum of approximately 3×10^6 cells ml^{-1} was prepared in 0.85% (w/v) sterile NaCl solution.

To examine the ability of the obtained isolates in dissipating NS and select the pre-eminent degrader(s), bacterial suspensions of each were added to flasks (approximately 3×10^6 cells ml^{-1}) containing 50 ml BM + NS (20 μg ml^{-1}) in triplicate. Triplicated non-inoculated media of BM + NS were also prepared as controls. All the flasks were incubated in the dark at 30°C and 150 rpm. After 10 days, subsamples of the well-mixed cultures were taken to evaluate the dissipation of NS. One isolate (B9) with the ability to dissipate more than 50% of NS in 10 days was selected for further experiments.

The B9 isolate was identified through 16S rRNA gene partial sequencing. Details on DNA extraction and sequencing procedures are provided in SI section D. The 16S rRNA gene sequence of the B9 isolate was submitted in GenBank database with the accession number of MG966184.

Bioassay tests for NS

To evaluate the sensitivity of the test plant to NS and select appropriate herbicide concentrations for the detoxification tests, bioassay experiments were conducted using a local variety of Cress (*Lepidium sativum* L.). Prior to bioassay, the seeds of *L. sativum* were sterilized by washing in 96% ethanol for 30 sec followed by 1.5–2 min immersion in a 10% sodium hypochlorite solution. The seeds were then rinsed 7–8 times with sterile distilled water and placed in sterile Petri dishes containing 10 g l^{-1} agar in water. The plates were incubated at 20 ± 3°C for 24 h until germination of the seeds.

Bioassay experiments were performed in triplicate using 9-mm diameter plastic Petri dishes as described by Tal *et al.* (2000). A pretest performed with NS (20 μg ml^{-1}) on the *L. sativum* seeds showed that the Cress shoots were more sensitive to the herbicide compared to the roots (data not shown). Therefore, shoot growth inhibition (SGI) was measured as the standard parameter in further bioassay and detoxification tests. Pregerminated seeds of *L. sativum* were placed at the bottom of the Petri dishes (20 in each Petri dish) between two layers filter papers (Whatman #1). Various concentrations of NS ranging from 0.2 to 1000 μg ml^{-1} were added to the Petri-dishes (7 ml). The dishes were incubated (16 h light: 25 ± 3°C; 8 h dark: 20 ± 3°C), and the lengths of the shoots were measured after 7 days. The effective concentration of NS causing 50% SGI for *L. sativum* (EC_{50}) was estimated through dose-response curves.

Dissipation and detoxification of NS in liquid media by the B9 isolate

The ability of the B9 isolate in dissipating NS, effects of additional sources of carbon on the NS decline, and changes in pH were investigated in liquid media. Triplicate

experiments were conducted in 500 ml Erlenmeyer flasks containing 100 ml of BM + NS, BM + NS + 0.25% Glucose (BM + NS + G), BM + NS + 0.05% Yeast extract (BM + NS + Y), and BM + NS + 0.25% Glucose + 0.05% Yeast extract (BM + NS + G + Y). Two sets of each medium were spiked with NS separately at three concentrations of 40 (equivalent to the EC_{50}), 80, and 160 μg ml^{-1}. One set of the spiked media, named B9 (+), was inoculated with the bacterial suspension of the B9 isolate at approximately 3×10^6 cells ml^{-1}, and the other set, named B9 (-), was left uninoculated. Triplicate non-spiked B9 (+) or B9 (-) flasks were also prepared as controls. The samples were incubated in the dark at 30°C and 150 rpm. At days 0, 2, 4, 6, and 10, subsamples were taken from each medium for pH and NS concentration measurements.

The potential of the B9 isolate in reducing the phytotoxicity of NS was tested using *L. sativum* seeds in Petri dishes. From each dissipation test medium, a well-mixed subsample was taken after 10 days of incubation, centrifuged (10000 rpm, 10 min), filtered (0.45 μm, Hydrophilic PTFE, Millex®, USA), and 7 mL was transferred to separate Petri dishes containing 20 seeds of *L. sativum*. All the dishes were incubated (16 h light: 25 ± 3°C; 8 h dark: 20 ± 3°C), and the lengths of the shoots were measured after 7 days. Finally, the SGI was measured in each experiment relative to the non-spiked controls.

Dissipation and detoxification of NS in soil by the B9 isolate

The experiments were performed with the same soils from which the B9 isolate was enriched but without any previous pesticide treatment history. Two sets of non-sterile soil samples (500 g) were spiked with the filter-sterilized aqueous solution of NS at 0.3 μg g^{-1} (field recommended dosage) and 15 μg g^{-1} (50 times the field recommended dosage) in glass containers. One set of the samples, named B9 (+), was then inoculated with the suspension of the B9 isolate at 3×10^6 cells g^{-1}, and the other one, named B9 (-), was left uninoculated. Non-spiked B9 (+) or B9 (-) soil samples were also prepared as controls. All the experiments were prepared in three replicates. After adjusting the moisture content of the samples to 80% of the MWHC with sterile distilled water and mixing, they were incubated in the dark at 30°C for 30 days. During the incubation period, moisture was maintained by adding distilled water. At 0, 7, 14, 21, and 30 days during incubation, NS concentration was measured in each sample.

The NS detoxification ability of the B9 isolate in soil was evaluated using standard-sized pots (10 cm ID × 10 cm length). Soil subsamples (200 g) from each of the above experiments at day 30 were transferred into the pots. Pregerminated seeds of the test plant were sown in each pot at a depth of 1.5 cm (10 seeds per each pot). The pots were incubated at the conditions of 16 h light: 25 ± 3°C and 8 h dark: 20 ± 3°C with a moisture content of 80% MWHC. After 14 days, the plants in each pot were uprooted, and the SGI was measured relative to the non-spiked controls.

Soil microbial activity

The activity of the soil microorganisms during the dissipation experiments of NS was evaluated by taking subsamples from each treatment at 0, 7, 14, 21, and 30 days during incubation. SR was measured based on the procedure specified by Froment (1972). For this, 20 g of the soil samples (55% MWHC moisture) were placed in 500 ml containers

containing a small vessel with 20 ml of 0.2 N NaOH. Same containers without any soil were also prepared as controls. All the samples were incubated at 30°C in the dark for 10 days. The CO_2 trapped by NaOH was determined by titration with 0.1 N HCl. Results were reported in mg CO_2 g^{-1} soil per 24 h. MBC was measured with the chloroform fumigation-extraction method defined by Jenkinson *et al.* (1979). Soils were first fumigated with alcohol-free chloroform ($CHCl_3$) under moist conditions for 24 h. Then, carbon contents of the soils were extracted with 0.5 M potassium sulfate (K_2SO_4) and measured by titration with a 0.0333 M solution of Mohr salt ($(NH_4)_2Fe(SO_4)_2$) in the presence of the phenylanthranilic acid as the indicator. Non-fumigated samples were extracted and measured under equal conditions as controls. The MBC was estimated based on the differences between the carbon content of the fumigated and non-fumigated soil adopting a factor of 0.38 (Vance *et al.*, 1987). Each microbial endpoint was measured with five replicates, and the results were presented as μg C g^{-1} soil. DA of the soil samples was evaluated according to Tabatabai (1982). Soils were spiked with triphenyl tetrazolium chloride (TTC) under moist conditions and incubated at 37°C for 24 h. Triphenyl formazan (TPF), which is the product of TTC degradation by the dehydrogenase enzyme, was measured spectrophotometrically at the wavelength of 485 nm and the enzyme activity was described as μg TPF g^{-1} soil in 24 h.

NS chemical analysis

NS was extracted from the soils and liquid media by EtOAc and the method described by Pinto *et al.* (2010), and the cleanup was performed with PSA (Anastassiades *et al.* 2003). For liquid cultures, 1 mL of each medium was mixed with 2 mL EtOAc, and the extraction was achieved similar to the soil samples. Concentrations of NS were measured using the high-performance liquid chromatography (HPLC) equipped with a photodiode array detector (Platin Blue HPLC System, ChromeJo®). Separations were performed on a reverse-phase C_{18} column (250 mm × 4 mm ID; Knauer®, Germany). The mobile phase consisted of water + ACN + H_3PO_4 (20 + 80 + 0.1 (v/v)) at 1 ml min^{-1} and in an isocratic elution mode (Ahmadi *et al.*, 2017). Each sample was injected (15 μl) three times. The extraction and analysis methods were validated in terms of instrumental linearity, method accuracy, and precision according to standard guidelines established by the European Commission (2006). Details on the validation procedures are described elsewhere (Torabi *et al.*, 2017), and the results are presented in SI section E.

Data analysis

The EC_{50} for NS was calculated by fitting the dose-response data to a log-logistic model (Seefeldt *et al.*, 1995) using GraphPad Prism 7. The dissipation kinetics and degradation times for NS in liquid media were calculated using a simple first-order kinetic (SFOK) model according to the instructions provided by to the North American Free Trade Agreement (NAFTA) guidance (US-EPA; https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/guidance-evaluating-and-calculating-degradation).

Results and discussion

Identification of the B9 isolate

Results of 16S rRNA gene sequencing and homology searches within the GenBank database revealed a 96% resemblance of the B9 isolate to *P. aeruginosa* strains (Figure S1). This is also in agreement with colony characteristics of the B9 isolate (Table S3). *P. aeruginosa* is a Gram-negative, rod-shaped, and aerobic bacterium and has been proved to degrade pesticides such as parathion and endosulfan (Senthilkumar *et al.*, 2011), malathion (Thabit and El-Naggar, 2013), acephate and methamidophos (Ramu and Seetharaman, 2014), and chlorpyriphos (Fulekar and Geetha, 2008). Furthermore, *Pseudomonas* sp. has been shown to be capable of degrading sulfonylurea herbicides including chlorimuron-ethyl (Ma *et al.*, 2009) and ethametsulfuron-methyl (Li-Feng *et al.*, 2007).

Seed bioassay

Results of seed bioassay experiments are represented in Table 2 and Figure S2. According to these, different concentrations of NS inhibited the growth of *L. sativum* shoots, and the EC_{50} of the herbicide was 40 μg ml^{-1}. Previous studies have revealed the high phytotoxicity of NS and other sulfonylurea herbicides, that is, sulfosulforun, for *L. sativum* (Gassam *et al.*, 2010; Kazemi and Hoodaji, 2017). Also, *L. sativum* has been used as a sensitive plant in bioassay tests of hexazinone and simazine (Ortega *et al.*, 2004).

Dissipation and detoxification of NS by the B9 isolate in liquid media

Liquid culture tests, in general, showed significantly faster dissipation of NS in the B9 (+) media compared to the B9 (-) ones (Table 3). In the B9 (+) cultures, the dissipation rate of NS was significantly faster at 40 μg ml^{-1} compared to 80 or 160 μg ml^{-1} (Table 4). This is probably due to higher toxic effects of NS at 80 and 160 μg ml^{-1} compared to 40 μg ml^{-1} resulting in inhibition of the isolate's degradation efficiency. Similarly, Zhang *et al.* (2012) and Wang *et al.* (2016) showed the inhibition of NS degradation by *Serratia marcescens* N80 and *Klebsiella* sp. Y1, respectively, as a result of an increase in the initial concentration of the herbicide.

In BM + NS + G and BM + NS + G + Y media with NS at 40 μg ml^{-1}, the removal rate of the herbicide enhanced by approximately 2 and 3 times, respectively, compared to BM + NS and coincided with a significant drop in the pH (< 5) after 10 days (Tables 3–5). At 80 and 160 μg ml^{-1} also, the dissipation of NS in the presence of glucose or yeast extract was approximately up to 1.5 to 3 times faster compared to BM + NS, and a significant reduction

Table 2. Log-Logistic model parameters for seed bioassay of NS on *L. sativum*

					EC_{50}		
Minimum value ± SE	Maximum value ± SE	Slope ± SE	r^2	Log EC_{50} ± SE	Value (μg ml^{-1})	LCI (μg ml^{-1})	UCI (μg ml^{-1})
4.69 ± 1.79	105.41 ± 8.43	2.44 ± 0.30	0.98	1.60 ± 0.13	39.81	21.88	72.44

LCI: lower confidence interval 95%, **UCI**: upper confidence interval 95%, **SE**: standard error obtained from the regression analysis of the log-logistic model.

Table 3. Dissipation and detoxification of NS in liquid media

		BM + NS				BM + NS + G				BM + NS + G + Y			
		B9 (+)		B9 (-)		B9 (+)		B9 (-)		B9 (+)		B9 (-)	
SC (μg ml^{-1})	Days	D (%) ± SE	SGI (%) ± SE	D (%) ± SE	SGI (%) ± SE	D (%) ± SE	SGI (%) ± SE	D (%) ± SE	SGI (%) ± SE	D (%) ± SE	SGI (%) ± SE	D (%) ± SE	SGI (%) ± SE
40	0	0 ± 1	45 ± 3	0 ± 0	58 ± 4	0 ± 1	38 ± 3	0 ± 0	55 ± 3	0 ± 2	30 ± 2	0 ± 1	54 ± 2
	2	14 ± 5		0 ± 5		19 ± 4		0 ± 1		33 ± 8		0 ± 2	
	4	23 ± 6		1 ± 3		37 ± 6		2 ± 3		50 ± 7		2 ± 1	
	6	32 ± 4		2 ± 2		52 ± 8		5 ± 1		61 ± 6		3 ± 4	
	10	39 ± 7		4 ± 3		65 ± 7		6 ± 4		75 ± 4		4 ± 3	
80	0	0 ± 2	60 ± 4	0 ± 0	65 ± 3	0 ± 1	63 ± 2	0 ± 0	66 ± 2	0 ± 0	54 ± 4	0 ± 0	64 ± 6
	2	6 ± 5		1 ± 3		9 ± 3		1 ± 2		21 ± 5		0 ± 1	
	4	14 ± 5		2 ± 4		17 ± 5		3 ± 1		31 ± 7		0 ± 2	
	6	22 ± 4		4 ± 2		25 ± 6		4 ± 3		45 ± 9		2 ± 1	
	10	27 ± 5		5 ± 3		30 ± 5		5 ± 3		56 ± 4		3 ± 3	
160	0	0 ± 1	70 ± 5	0 ± 0	73 ± 5	0 ± 0	69 ± 3	0 ± 0	74 ± 4	0 ± 1	65 ± 6	0 ± 0	75 ± 2
	2	12 ± 7		1 ± 2		6 ± 4		2 ± 2		13 ± 4		0 ± 2	
	4	17 ± 8		3 ± 1		13 ± 7		3 ± 1		24 ± 6		0 ± 1	
	6	23 ± 6		4 ± 3		19 ± 6		6 ± 3		31 ± 3		1 ± 2	
	10	30 ± 5		6 ± 1		29 ± 5		8 ± 3		46 ± 5		2 ± 1	

SC: spiking concentration, **D**: dissipation, **SGI**: shoot growth inhibition calculated relative to the non-spiked controls (media without nicosulfuron), **BM + NS**: base medium plus nicosulfuron, **BM + NS + G**: base medium plus nicosulfuron and glucose (0.25%), **BM + NS + G + Y**: base medium plus nicosulfuron, glucose (0.25%), and yeast extract (0.05%), **B9 (+)**: medium plus the B9 isolate (3×10^6 cells ml^{-1}), **B9 (-)**: medium without the B9 isolate, **SE**: standard error of triplicate measurements.

Table 4. SFOK model parameters and dissipation times for SN in liquid media or soil inoculated with the B9 isolate

		SFOK model parameters		Dissipation times	
Sample	SC (μg ml^{-1} or μg g^{-1})	r^2	k (day^{-1}) ± SE	DT_{50} (day) ± SE	DT_{90} (day) ± SE
BM + NS	40	0.95	0.05 ± 0.00	13 ± 2	44 ± 4
	80	0.95	0.03 ± 0.01	20 ± 3	67 ± 11
	160	0.94	0.04 ± 0.01	19 ± 1	64 ± 12
BM + NS + G	40	0.99	0.11 ± 0.01	6 ± 1	20 ± 2
	80	0.97	0.04 ± 0.00	18 ± 1	60 ± 4
	160	0.98	0.03 ± 0.00	20 ± 1	66 ± 3
BM + NS + G + Y	40	0.92	0.16 ± 0.03	4 ± 1	15 ± 3
	80	0.98	0.09 ± 0.01	8 ± 1	26 ± 4
	160	0.98	0.06 ± 0.00	11 ± 1	37 ± 2
nSS + NS + B9	0.3	0.99	0.08 ± 0.00	8 ± 1	28 ± 2
	15	0.97	0.03 ± 0.00	21 ± 5	70 ± 17
nSS + NS – B9	0.3	0.97	0.03 ± 0.00	27 ± 5	76 ± 18
	15	0.97	0.02 ± 0.00	37 ± 6	124 ± 19

SC: spiking concentration, **D**: dissipation, **BM + NS**: base medium plus nicosulfuron, **BM + NS + G**: base medium plus nicosulfuron and glucose (0.25%), **BM + NS + G + Y**: base medium plus nicosulfuron, glucose (0.25%), and yeast extract (0.05%), **nSS + NS + B9**: non-sterile soil plus nicosulfuron and the B9 isolate (3×10^6 cells g^{-1}), **nSS + NS – B9**: non-sterile soil plus nicosulfuron without the B9 isolate, **SE**: standard error obtained from the regression analysis of the SFOK model.

Table 5. Changes in the pH of the media during NS dissipation

		pH ± SE					
		BM + NS		BM + NS + G		BM + NS + G + Y	
SC (μg ml^{-1})	Days	B9 (+)	B9 (-)	B9 (+)	B9 (-)	B9 (+)	B9 (-)
40	0	7.21 ± 0.02	7.22 ± 0.06	7.20 ± 0.03	7.19 ± 0.03	7.20 ± 0.04	7.21 ± 0.07
	2	7.01 ± 0.04	7.22 ± 0.03	6.45 ± 0.04	7.15 ± 0.03	6.21 ± 0.03	7.18 ± 0.04
	4	6.23 ± 0.05	7.18 ± 0.04	5.85 ± 0.05	7.12 ± 0.02	5.43 ± 0.05	7.10 ± 0.05
	6	5.65 ± 0.03	7.10 ± 0.03	5.12 ± 0.02	7.07 ± 0.01	4.99 ± 0.02	7.04 ± 0.06
	10	5.32 ± 0.05	6.93 ± 0.03	4.75 ± 0.04	6.99 ± 0.05	4.49 ± 0.08	6.98 ± 0.04
80	0	7.23 ± 0.06	7.21 ± 0.04	7.20 ± 0.03	7.19 ± 0.04	7.21 ± 0.01	7.22 ± 0.02
	2	6.95 ± 0.07	7.20 ± 0.03	6.52 ± 0.04	7.14 ± 0.06	6.32 ± 0.03	7.19 ± 0.03
	4	6.32 ± 0.05	7.18 ± 0.03	5.94 ± 0.03	7.10 ± 0.05	5.61 ± 0.04	7.14 ± 0.04
	6	5.92 ± 0.03	7.14 ± 0.02	5.35 ± 0.04	7.01 ± 0.04	5.12 ± 0.03	7.10 ± 0.05
	10	5.79 ± 0.03	7.10 ± 0.02	4.83 ± 0.01	6.97 ± 0.03	4.64 ± 0.05	7.09 ± 0.02
160	0	7.20 ± 0.02	7.22 ± 0.03	7.21 ± 0.03	7.20 ± 0.04	7.22 ± 0.03	7.23 ± 0.03
	2	7.00 ± 0.04	7.16 ± 0.01	6.66 ± 0.04	7.17 ± 0.03	6.51 ± 0.02	7.18 ± 0.05
	4	6.56 ± 0.05	7.12 ± 0.05	5.91 ± 0.03	7.15 ± 0.01	5.82 ± 0.06	7.14 ± 0.03
	6	6.01 ± 0.05	7.06 ± 0.03	5.41 ± 0.05	7.13 ± 0.03	5.38 ± 0.03	7.06 ± 0.04
	10	5.65 ± 0.02	6.95 ± 0.04	4.80 ± 0.06	7.05 ± 0.01	4.74 ± 0.04	6.96 ± 0.05

SC: spiking concentration, **D**: dissipation, **BM + NS**: base medium plus nicosulfuron, **BM + NS + G**: base medium plus nicosulfuron and glucose (0.25%), **BM + NS + G + Y**: base medium plus nicosulfuron, glucose (0.25%), and yeast extract (0.05%), **B9 (+)**: medium plus the B9 isolate (3×10^6 cells ml^{-1}), **B9 (-)**: medium without the B9 isolate, **SE**: standard error of triplicate measurements.

of the pH was noticed. Sulfonylurea herbicides have low stability under acidic conditions due to their low isoelectric point (Sabadie, 2002), and co-metabolic microbial degradation of glucose can lead to the production of acidic compounds such as acetic, oxalic, and lactic acids. Therefore, the presence of glucose can enhance the degradation of these groups of herbicides through microbial acidohydrolysis (Song *et al.*, 2013; Wang *et al.*, 2016; Zhao *et al.*, 2015). Previous studies have proved microbial acidohydrolysis for NS (Feng *et al.*, 2017; Wang *et al.*, 2016) and tribenuron methyl (Wang *et al.*, 2012). Wang *et al.* (2016) showed that in the presence of glucose and acidic conditions, acidohydrolysis initially results in the cleavage of the C-N bond of the sulfonylurea bridge of NS and production of 2-amino-4,6-dimethoxypyrimidine and 2-aminosulfony-N,N-dimethylnicotinamide.

These transformation products are however stable in the pH range of 3–7 and, hence, further biodegraded through microbial processes (Wang *et al.*, 2016).

Seed experiments in Petri dishes confirmed that the B9 isolate was able to detoxify NS and decrease the SGI considerably in liquid media (Table 3). The most distinguished detoxification was recognized in media with the highest dissipation rate of NS (BM + NS + G + Y plus NS at 40 μg ml^{-1}), and the isolate reduced the SGI significantly (SGI = 30 ± 2%) compared to the B9 (-) samples (SGI = 54 ± 2%). At 160 μg ml^{-1}, however, the differences between SGI in the B9 (+) and B9 (-) samples (65 ± 6 to 70 ± 5% and 73 ± 5 to 75 ± 2%, respectively) were not significant, despite a 29–46% reduction of the NS in the B9 (+) soils (Table 3). This reveals that the detoxification capacity of the isolate has reduced at higher NS concentrations.

Dissipation and detoxification of NS by the B9 isolate in soil

The dissipation of NS in soils followed the first-order pattern and was fitted to the SFOK model with rates between 0.02 and 0.08 day^{-1} (Table 4). Introducing the B9 isolate to non-sterile soils at 3×10^6 cells g^{-1} accelerated the dissipation of NS at both concentrations within 10 days (Tables 4 and 6). This shows that the applied inoculum density was sufficient to survive the initial competition with other indigenous micro-organisms present within a biotic and non-sterile soil. Inoculum concentration is considered as a key factor guaranteeing growth and degrading abilities of an isolate (Cycoń *et al.*, 2009; Rousseaux *et al.*, 2003; Singh *et al.*, 2006). It has been shown that introducing low densities of bacterial isolates (< 10^4 cells g^{-1} soil) to non-sterile soils can significantly decrease their survival degradation efficiencies (Cullington and Walker, 1999; Ramadan *et al.*, 1990).

In soils also, the presence of the B9 isolate at both concentrations decreased the SGI significantly compared to B9 (-) samples. Considering the percentage of SGI in B9 (-) samples, the reduction of SGI at 0.3 μg g^{-1} was approximately three times higher than 15 μg g^{-1}. This again demonstrates the better ability of the isolate to detoxify lower concentrations of NS and was also associated with 2.6 times faster dissipation rate of the herbicide compared to 15 μg g^{-1} (Table 6). Overall, results of detoxification experiments highlighted that the degradation of NS by the B9 isolate was characterized by a

Table 6. Dissipation and detoxification of NS in soil

		nSS + NS + B9		nSS + NS − B9	
SC (μg g^{-1})	Days	D (%) ± SE	SGI (%) ± SE	D (%) ± SE	SGI (%) ± SE
0.3	0	0 ± 7	2 ± 1	0 ± 6	8 ± 2
	7	45 ± 4		16 ± 9	
	14	70 ± 8		37 ± 9	
	21	81 ± 9		51 ± 6	
	30	88 ± 7		55 ± 7	
15	0	0 ± 5	19 ± 3	0 ± 3	26 ± 4
	7	26 ± 7		9 ± 6	
	14	42 ± 9		19 ± 9	
	21	53 ± 6		32 ± 12	
	30	59 ± 3		43 ± 4	

SC: spiking concentration, **D**: dissipation, **SGI**: shoot growth inhibition calculated relative to the non-spiked controls (soils without nicosulfuron), **nSS + NS + B9**: non-sterile soil plus nicosulfuron and the B9 isolate (3×10^6 cells g^{-1}), **nSS + NS – B9**: non-sterile soil plus nicosulfuron without the B9 isolate, **SE**: standard error of triplicate measurements.

notable loss of the herbicide's phytotoxicity in culture media and soils, particularly at lower concentrations. This can be the result of producing innocuous transformation products with less toxicity compared to the parent compound. Similarly, other researchers have confirmed the detoxification capacity of enriched bacterial cultures for pesticides such as endosulfan (Awasthi *et al.*, 2003; Kumar *et al.*, 2007) and pendimethalin (Elsayed and El-Nady, 2013).

Effect of NS on soil microbial activities

Effects of NS on soil microbial activity parameters are illustrated in Figure 1. SR and MBC are indicators of overall soil microbial activity and living organic matter, respectively (Dutta *et al.*, 2010; Radivojević *et al.*, 2012). DA is also regarded as a well-known sign of microbial metabolism and is associated with oxidation-reduction processes in soil (Stepniewska *et al.*, 2007). Therefore, shifts in these parameters are considered as good indicators for the effects of pesticides on soil microbial activities (Imfeld and Vuilleumier, 2012). The impacts of pesticides on soil microbial activities have been investigated by various researchers, and the results indicate that these effects are usually transient and can be inhibitory or stimulatory (Crouzet *et al.*, 2010; Cycoń and Piotrowska-Seget, 2009; Gomez *et al.*, 2009; Radivojević *et al.*, 2012; Wang *et al.*, 2010; Zabaloy *et al.*, 2008). Furthermore, the dependency of these responses to the initial concentration of an applied pesticide has been proved (Dungan *et al.*, 2003; Filimon *et al.*, 2015; Gomez *et al.*, 2009; Šantrić *et al.*, 2016). The effects of pesticides on the soil microbial activities can also vary depending on the bioavailable fraction of their molecules for the soil microorganisms. In this context, soil properties, that is, OM content, and characteristics of the pesticide molecule, that is, soil sorption coefficient (K_{oc}) and hydrophobicity, can regulate the bioavailability of a pesticide in soil and its effect on the microbial responses though sorption-desorption processes (Dungan *et al.*, 2003; Gevao *et al.*, 2000). As a result, soil microbial activities are usually affected stronger by the pesticides with a low K_{oc} and in soils with small OM contents (Sommerville and Greaves, 1987).

In case of our study, treating soils with both concentrations of NS initially decreased SR, MBC, and DA significantly compared to non-spiked samples, especially at 15 μg g^{-1} (Figure 1). NS has high water solubility and low K_{oc} (Table S1). Furthermore, our experimental soil contains low OM content (Table 1). Therefore, we can assume that the primary inhibition of soil microbial activities at both concentrations of NS was due to initial high bioavailability of the herbicide in the soil. Similarly, Radivojević *et al.* (2012) showed initial inhibition of soil microbial activities by NS at different concentrations ranging between 0.3 and 3 μg g^{-1}. Also, Filimon *et al.* (2015) and Šantrić *et al.* (2016) showed that NS could reduce the growth of *Clostridium* sp. and actinomycetes, respectively, in a dose-dependent manner and the highest inhibition was achieved at concentrations 7–50 times of the recommended field application. Furthermore, in our study, the inhibitory effects of SN on soil microbial activities were transient, and the biochemical parameters recovered subsequently during 30 days. This indicates that the populations of NS-adapted microorganisms might have become more abundant in the soils during the time (Crouzet *et al.*, 2010; Vandana *et al.*, 2012). Interestingly, the recovery of soil

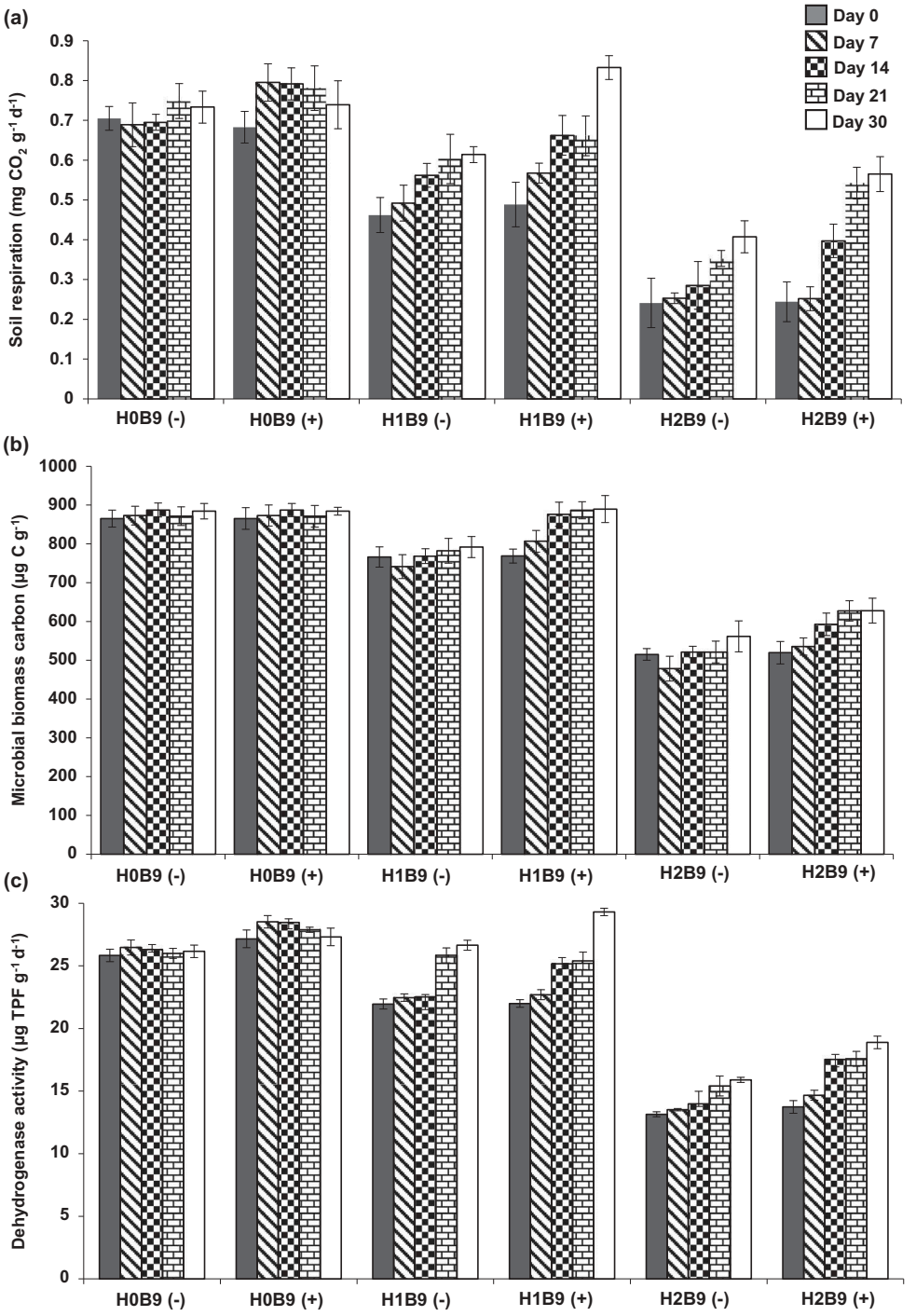

Figure 1. a) soil respiration, b) microbial biomass carbon, and c) dehydrogenase activity in different non-sterile soil treatments. **H0**: no herbicide, **H1**: NS at 0.3 µg g^{-1}, **H2**: NS at 15 µg g^{-1}, **B9 (-)**: without the B9 isolate, **B9 (+)**: plus the B9 isolate (3×10^6 cells g^{-1}). Error bars represent the standard deviations of three replicates

microbial activity was significantly faster in the B9 (+) samples. This reveals that the isolate was able to successfully colonize and increase the metabolic activity in the soil at the inoculated density.

Conclusion

In this research, a *P. aeruginosa* strain capable of degrading and detoxifying NS was isolated from an agricultural soil. In the liquid culture, the ability of the isolate in dissipating NS, especially at 40 μg ml^{-1}, and reducing the pH of the media was proved. Additional sources of energy such as glucose and yeast extract enhanced the pH decline and the dissipation rate of NS significantly probably as a result of microbial acidohydrolysis. The dissipation rate of NS and microbial activities in both B9 (+) and B9 (-) soils were dose dependent and reduced significantly at 15 μg g^{-1} compared to 0.3 μg g^{-1}. The presence of the B9 isolate accelerated the dissipation of NS in the soils significantly, especially at 0.3 μg g^{-1}. Exposure of the soils to NS at both concentrations reduced the microbial activities temporarily with a subsequent recovery after 30 days, and inoculation of the isolate speeded up this recovery. The degradation of NS by the isolate, especially at lower concentrations of the herbicide, resulted in a significant reduction of *L. sativum* SGI, which shows the ability of the bacterium in detoxifying the compound during the degradation process.

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Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflict of interest.

Informed consent: Informed consent was obtained from all individual participants included in the study.

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