

Differences in bacterial functional profiles from loamy sand and clay loam textured soils under fungicide Quadris^R impact

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Abstract

The non-target effect of the fungicide Quadris^R on the bacterial community from grassland loamy sand (LS) and cropland clay loam (CL) soils with unknown history of fungicide usage was investigated. Quadris^R was applied to soil mesocosms at 0.0 mg kg⁻¹ (Az0), 2.90 mg kg⁻¹ (Az1), 14.65 mg kg⁻¹ (Az2) and 35.0 mg kg⁻¹ (Az3) calculated towards the active ingredient azoxystrobin (Az). Response of bacterial communities to Quadris^R was investigated during a 120-day incubation experiment, evaluating the shifts in bacterial catabolic profiles by the community-level physiological profiling (CLPP) technique and Biolog EcoPlatesTM method. Quadris^R decreased the overall catabolic activity (AWCD) of soil bacterial communities and the rate of decrease was independent of soil type and fungicide concentration. Fungicide affected negatively the utilisation of amines and positively that of amino acids in both soil types, whereas the effects on other carbon guilds (carbohydrates, carboxylic acids and polymers) corresponded closely to the respective soil type and fungicide concentration. Results indicated the presence of non-target effects of Quadris^R on bacterial functioning; hence, it is important to address the fungicide side-effects on soil health.

Keywords

Average well colour development, community-level physiological profiling, fungicide azoxystrobin, Quadris^R, soil bacterial communities

Introduction

Plant diseases are a common occurrence, often having a significant economic impact on yield and quality; thus, managing diseases is an essential component of production for most crops. For this reason, fungicides are used to kill fungi by damaging their cell membranes, inactivating critical enzymes or proteins or by interfering with key processes, such as energy production or respiration (McGrath 2004). One of the widely used classes of fungicides is strobilurins (Howell et al. 2014). The first patent for a strobilurin fungicide (azoxystrobin) was introduced in 1996 (Bartlett et al. 2002) and, subsequently, a series of strobilurin fungicides (pyraclostrobin, fluoxastrobin, kresoxim-methyl, trifloxystrobin, picoxystrobin, mandestrobin and metominostrobin) were developed and marketed (Rodrigues et al. 2013). Strobilurin fungicides specifically bind to the quinol oxidation (Q_o) site of cytochrome b and inhibit mitochondrial respiration (Bartlett et al. 2002). Strobilurin fungicides control an unusually wide array of fungal diseases, including diseases caused by water moulds, downy mildews, powdery mildews, leaf spotting and blighting fungi, fruit rotters and rusts (Vincelli 2002). They are used on a wide variety of crops, including cereals, field crops, fruits, tree nuts, vegetables, turf-grasses and ornamentals.

These pesticides are designed to manage fungal pathogens, although their broad-spectrum mode of action also produces non-target impacts. Due to the unique mechanism of action, strobilurins may directly affect soil fungi by inhibiting mitochondrial respiration, inducing a shift from fungal to bacterial dominance in soil activities (Baćmaga et al. 2015). Strobilurins may affect not only soil fungi, but also soil bacteria (Baćmaga et al. 2015), archaea (Howell et al. 2014) and invertebrates (Han et al. 2014). For instance, Baćmaga et al. (2015) reported negative effects of azoxystrobin not only on soil fungi, but also on soil organotrophic bacteria and actinomycetes. Most of the earlier studies reported low to negligible effects of Az alone or Az containing fungicides on bacterial diversity, but the knowledge of fungicide effects on bacterial metabolic activity is still insufficient.

The aim of this study was to elucidate the effects of Quadris^R, Az containing fungicide, on soil bacterial metabolism. The study suggested that Quadris^R can potentially cause long-term adverse effects on soil nutrient turnover, affecting bacterial metabolism, although bacteria are considered as fungicide non-target organisms.

Material and methods

Sampling site and preliminary soil preparation

In this study, two soils with different histories of management practice were used. Five subsamples were pooled randomly from the surface layers (0–20 cm) of grassland and cropland located near Gabra Village (Sofia Region, Bulgaria): 42°31'48.36"N, 23°37'28.20"E (Fig. 1). The subsamples per soil type were sieved through a 2 mm mesh and mixed in aliquots after determining the dry weights of 1 g sample at 105 °C in an oven for 24 hr.



Figure 1. Map of Gabra Village territory (red line and red mark) and the sampling site (blue mark).

Mesocosm experimental design

Four sets of three replicated mesocosms (2 kg) were prepared for each soil type. The following treatments were studied: control (Az0) and Quadris^R amendments of 2.90 mg kg⁻¹ (Az1), 14.65 mg kg⁻¹ (Az2) and 35.00 mg kg⁻¹ (Az3), calculated towards the active ingredient – Az. Soil water content was adjusted to 60% of the maximum water holding capacity and it was maintained with sterile distilled water during the experiment. The mesocosms were incubated at 22 ± 1 °C in dark to prevent physical degradation of Az by light. Soil samples were collected randomly in triplicates from each mesocosm on the 1st (D1), 30th (D30), 60th (D60), 90th (D90) and 120th (D120) day after fungicide application.

Soil physico-chemical properties

Soil texture was defined and classified according to ISO 11277 (2009) and SSDS (1993), respectively. Soil pH was measured potentiometrically (HANNA Instruments) after mixing soil in 0.01 mol l⁻¹ CaCl₂ solution and shaking for 30 min (1:5; weight: volume). Soil nitrate (NO₃-N) and ammonium (NH₄-N) nitrogen and phosphates (P₂O₅) were determined spectrophotometrically, according to the methods of Keeney and Nelson (1982) and Olsen (1982), respectively.

Az residues in soil

The method of Az soil residues extraction and determination was explained in detail in Aleksova (2020). Recovery rates of Az in each sample were satisfactory at 80.0%–85.0%. Modelling of Az dissipation in soils was conducted according to the recommendations of the FOCUS group (2006), using an Excel file (FOCUS_DEGKIN V2) provided online by the group. The same file was used to calculate the time of 50% reduction in Az soil concentrations (DT50).

Community level catabolic activity and physiological profiling

EcoPlates (Biolog Inc., Hayward, CA, USA) were used to establish the changes in CLPPs over time. The procedure of plates' inoculation, cultivation and monitoring (every 12 hr for 5 days) was described in detail in Kenarova et al. (2014). During the initial data processing, the control OD was subtracted from the OD of each carbon source (CS) well and the CSs with corrected OD < 0.25 were considered as non-oxidised and their values were set to zero (Garland 1996). Biolog CSs were grouped according to Weber and Legge (2009) into five carbon guilds (CGs) depending on their chemical moieties: carbohydrates (CH; 10 CSs), polymers (Polym; 4 CSs), carboxylic acids (CA; 9 CSs), amino acids (AA; 6 CSs) and amines/amides (Amin; 2 CSs). The Biolog-derived data were used to evaluate the bacterial metabolic activity (AWCD) (Garland and Mills 1991) and the pattern of CLPP (Kenarova et al. 2014).

Data analysis

Each data point in the paper represented the mean value of the respective Az soil amendment \pm standard deviation. One-way ANOVA, followed by Tukey's test, was performed to examine the differences in the means of soil (pH, NO₃-N, NH₄-N, P₂O₅, Az) and bacterial (AWCD and CLPP) parameters. Principal component analysis (PCA) was performed with soil abiotic data to assess the differences in soil physical environments after Quadris^R application. The differences in CLPPs between soil types and amongst fungicide concentrations were assessed with the graph 'one-to-one' technique. The above statistics were performed with the package PAST (Hammer et al. 2001) at a level of significance $p < 0.05$.

Results

Soil environments

The soil textures of grassland and cropland were classified as loamy sand (LS; 2% clay, 15% silt and 83% sand) and clay loam (CL; 27% clay, 37% silt and 36% sand), respectively. Soils were well abundant in organic carbon (LS: 21.92 ± 1.41 g kg⁻¹ and CL: 23.4 ± 3.11 g kg⁻¹) and Kjeldahl nitrogen (LS: 2.20 ± 0.21 g kg⁻¹ and CL: 2.64 ± 0.34 g kg⁻¹), both of them fluctuating insignificantly during the incubation time. Soil pH was moderately acidic (5.63) at LS and neutral (6.99) at CL and, during the incubation, it decreased significantly (LS: by Az1 – 8%, Az2 – 12% and Az3 – 14%) and insignificantly (CL: by Az1 – 0.8%, Az2 – 1.1% and Az3 – 1.3%) in fungicide amended soil mesocosms. Quadris^R application increased the overall soil NO₃-N – in LS by 10% (Az1), 13% (Az2) and 20% (Az3) and, in CL, by 70% (Az1), 34% (Az2) and 19% (Az3). On the other hand, the overall soil NH₄-N concentrations decreased by 15% (LS) and 39% (CL). Soil concentrations of P₂O₅ were much more

stable than those of the inorganic nitrogen, decreasing during the incubation by 8.6% (LS) and 14.3% (CL).

Az soil residues decreased over time and the rate of decrease was higher for LS than those for CL – DT50 ranged for LS from 36.5 ± 7.1 (Az1) to 86.6 ± 4.1 (Az3) days, whereas those for CL ranged from 130.8 ± 7.8 (Az1) to 212.1 ± 3.2 (Az3) days.

PCA, based on soil physico-chemical properties and Az soil residues, was conducted in order to elucidate the similarity amongst soil physical environments (Fig. 2) and the analysis indicated: 1) the respective LS and CL mesocosms differed significantly from each other, except Az1 where fungicide input approximated to the physical environments of LS and CL on D90; 2) significant differences within-soil physical environments were detected, except those of Az1 and Az2 at LS on D60; 3) temporal fluctuations of CL physical environments were smaller than those of LS.

Bacterial metabolic activity

The AWCD of Az0 (CL) -1.69 OD was calculated to be around 35% higher than that of Az0 (LS) -1.25 OD. Quadris^R application decreased the overall mean value of AWCD (except Az1 at LS and Az2 at CL) and the changes were significant (Az3 at

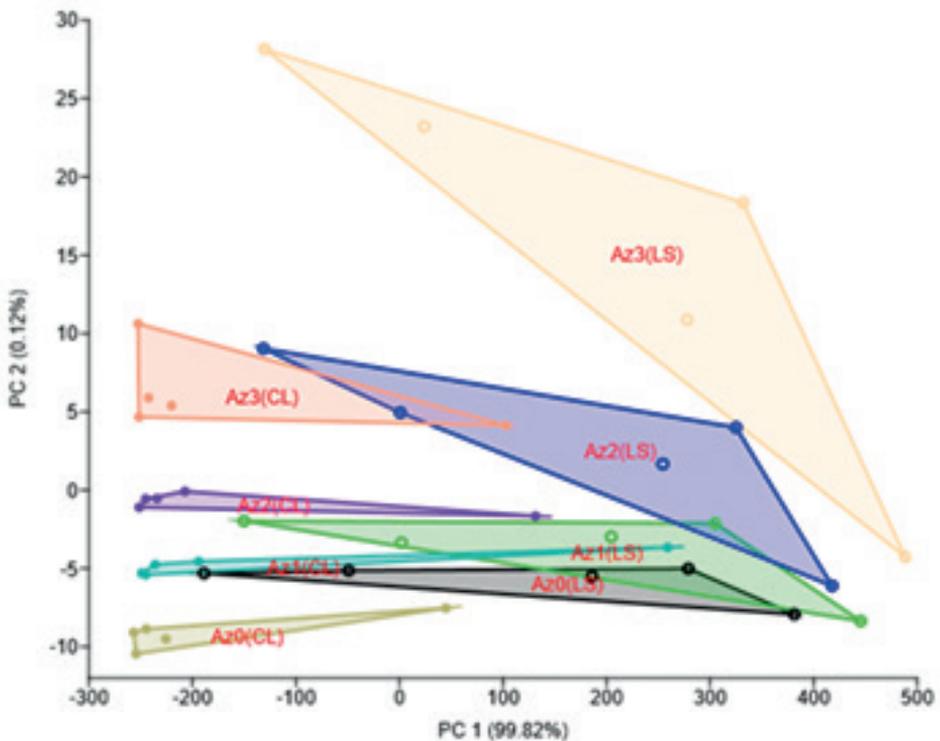


Figure 2. Spatial projection of the first two principal components (PC 1 and PC 2) with an ordination plot related to soil physical environments of Quadris^R amended (Az1 – Az3) and un-amended (Az0) loamy sand (LS) and clay loam (CL) soil mesocosms.

LS and Az1 and Az3 at CL) and insignificant (Az2 at LS). A stimulation effect was recorded for Az1 (LS) and Az2 (CL), being significant only for the second one. Temporal Quadris^R effects on bacterial metabolism were very similar, independent both on fungicide concentration and soil type – AWCD profiles manifested a decrease in bacterial metabolism for at least two months (D1 – D60), followed by recovery (D60 – D90) and stimulation (D120). Different metabolic profiles over time were formed for Az2 and Az3 at CL – permanent stimulation (Az2, except on D1) and dramatic decrease (Az3 after D60) after fungicide application. The values of Quadris^R that influenced AWCDs were much higher than that of Az0 on D120 (except Az3 at CL) and the rates of stimulation were in reverse- (LS) and non- (CL) relationships with the applied fungicide concentrations.

One-way ANOVA showed that the respective AWCD means of Az1 and Az3 did not differ significantly between LS and CL ($F < 2.05$, $p > 0.16$), opposite to that of Az2 ($F = 18.5$, $p = 0.000$).

Community level physiological profiling

It was obvious that bacterial metabolism was changed under Quadris^R impact, but AWCD was not sufficiently powerful to demonstrate the differences of these changes as a dependence of the applied fungicide concentration and soil properties. Therefore, after grouping the EcoPlate carbon sources into carbon guilds (CG), the CLPP approach and ‘one-to-one’ analysis were used to elucidate the intrinsic nature of AWCD changes (Fig. 3). Between-soil analysis indicated that, after Quadris^R, LS differed from CL by the utilisation of: 1) CH and CA – Az1, 2) all CGs, except CH – Az2, and 3) all CGs – Az3. Within-soil ‘one-to-one’ analysis demonstrated the effects of increasing fungicide concentrations on the utilisation of CGs in the respective soil type and they were significant at: 1) LS – all fungicide concentrations influenced the utilisation of CH (positively at Az1 and Az2 and negatively at Az3) and Amin (negatively at Az1 – Az3); Az1 and Az2 stimulated the utilisation of AA; Az3 decreased the utilisation of Polym and 2) CL - all fungicide concentrations influenced the utilization of-Amin (positively at Az2 and negatively at Az1 and Az3); Az1 and Az2 stimulated the utilisation of AA and CA (except Az1); Az3 decreased the utilisation of CH and Polym.

Metabolic diversity

In order to understand the insights of changes in CG utilisation rates under Quadris^R, the overall number of utilisable CSs were counted – metabolic richness, as well as the index of carbon sources’ utilisation evenness per CG.

Most of the changes occurred in CGs related to shifts into the “evenness” rather than the “richness” of utilisable CSs. For example, the richness changes under Quadris^R were detected only for the utilisation of CHs and CAs at CL - utilisation of carbohydrate D-Xylose (Az1 – Az3) was inhibited, whereas that of carboxylic acids, γ -Hydroxybutyric acid (Az1) and 2-Hydroxy benzoic acid and α -Ketobutyric acid (Az2 and Az3), was

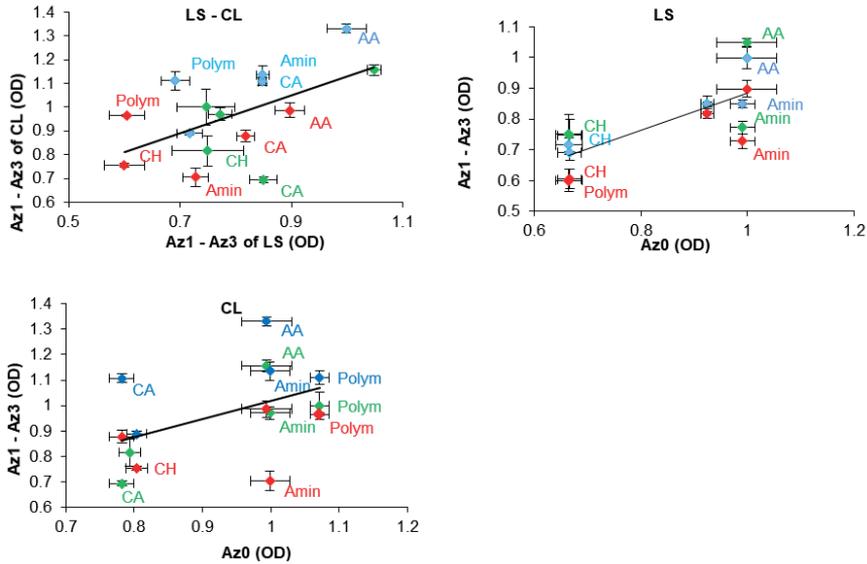


Figure 3. ‘One-to-one’ comparison of CLPPs between loamy sand and clay loam (LS – CL) soil mesocosms and between Quadris^R un-amended (Az0) and fungicide-amended soil mesocosms (Az1 – Az3) per soil type. Diamond symbols illustrate the mean ($n = 3$) utilisation rate of the respective carbon guild, bars illustrate the standard deviations and colour denotes the fungicide concentration - Az1: green, Az2: blue and Az3: red.

stimulated. Much more changeable amongst soil mesocosms was the index of metabolic evenness. The greatest between-soil type differences were detected at: 1) Az1, where Quadris^R increased the metabolic evenness of Polym, CA and Amin at LS and decreased that at CL and 2) Az3, where Quadris^R increased the metabolic evenness of CH, Polym and CA at CL and decreased that at LS. The between-soil type similarity was found at Az2, where Quadris^R increased the metabolic evenness of CA, AA and Amin.

Discussion

Soil physical environment

Soil amendments with Quadris^R, even by the lowest fungicide concentration, created new physical environments referring mainly to changes in soil pH, nitrogen pool and presence of allochthonous substrates (Az and Quadris^R's adjuvants). Similar soil acidification after Az application was also reported by earlier studies (Ghosh and Singh 2009; Singh et al. 2010), explaining this fact by the formation of azoxystrobin acid as the major product of fungicide degradation. In this study, the decrease in pH was recorded immediately after fungicide application (D1), assuming that azoxystrobin acid was not the only determinant of soil acidification. Probably, some of

the Quadris^R ingredients contributed also to soil acidification. We supposed that soil acidification might influence directly and/or indirectly bacterial metabolism, shifting community composition into growth of acidophiles and influencing nutrient solubility (bioavailability).

Soil amendments with Quadris^R influenced the soil nitrogen pool, changing bioavailable concentrations and forms of inorganic nitrogen which could be related to the adjuvants presented in fungicide commercial formulations (Devare et al. 2007; Mijangos et al. 2009), fungicide metabolisation (Cycoń et al. 2011; Baćmaga et al. 2017) and accumulation and degradation of proteins released from killed soil inhabitants (Wu et al. 2014; Zhang et al. 2014). Since nitrification and mineralisation play important roles in nutrient turnover (Edwards et al. 1995), it seems that shifts in soil inorganic nitrogen could disrupt these processes and impact overall soil quality and productivity. Additionally, proportions of soil NO₃-N and NH₄-N could also influence the utilisation rates of nitrogen containing CSs. We supposed that Az was more bioavailable in LS compared to CL and it influenced its persistence, half-life and toxicity to soil organisms. According to DT50, Az could be considered as a low to medium persistent fungicide in LS and highly persistent in CL.

The ordination of soil physical environments demonstrated that Quadris^R application in increasing concentrations influenced soil properties, creating new physical environments. We supposed that newly-created environments might influence soil bacteria to adapt their metabolism.

Fungicide effects on soil bacteria

In this study, we evaluated the Quadris^R effects on soil heterotrophic bacteria, which display a substantial role in plant growth rates, mineralising dead organic matter and detoxifying a range of exogenous substances. Bacteria are considered as Az non-target organisms, due to the fungicide mode of action on mitochondrial respiration (Bartlett et al. 2002). In fact, earlier reported data, referring to bacterial community composition (Howell et al. 2014) and functioning (Sułowicz et al. 2016; Wang et al. 2020) under Az, were very contradictory. We hypothesised that fungicides influenced soil bacterial metabolism indirectly by changes in soil biotic and abiotic properties. Two main metabolic criteria were followed during the soil mesocosms' incubation: 1) community metabolic activity expressed by AWCD and 2) community metabolic profiles (CLPP) expressed by carbon guilds' utilisation rates and metabolic diversity. In the case of detected fungicide impacts, it was important to mention if there were any relationships to soil type and, in particular, with some of the studied soil properties.

Community metabolic activity (AWCD)

Quadris^R application influenced bacterial metabolism for at least four months, decreasing it in most of the soil mesocosms, except at Az1 (LS) and Az2 (CL). The most serious negative effects were detected during the first two months after fungicide application,

followed by recovery and stimulation of bacterial metabolic activity (except Az3 at CL). Some researchers reported inhibitory effects of fungicides on bacterial metabolic activity (Zhang et al. 2014), although others advocated none or stimulation effects on AWCD (Muñoz-Leoz et al. 2011). We suppose that these contradictions arise from the fungicide chemical origin, applied concentrations and soil properties. In our study, soil properties were of significant importance for the differentiation of Quadris^R effects on overall AWCD at Az2, but not at the other fungicide concentrations. Probably, the delayed stimulation effects at Az1 decreased the differences in AWCD between LS and CL, whereas the very high value of Az3 minimised the modulating effects of soil peculiarities on fungicide mode of action.

Community level physiological profiles (CLPP)

Carbon guilds' utilisation rates

Earlier studies (Bending et al. 2007) and our investigations (Aleksova et al. 2021) reported that azoxystrobin did not affect bacterial community structure, suggesting that the fungicide shifted bacterial metabolism via chemical toxicity and/or phenotypic bacterial adaptation to environmental changes. The dissimilarity between LS and CL under Quadris^R, applied at a field recommended concentration, was referred towards CH and CA utilisation. These results confirmed the findings of some authors (Kenarova et al. 2014; Yu et al. 2020) that the utilisation of carbohydrates and carboxylic acids was sensitive to environmental disturbance and it could be used to indicate the alterations that occurred in bacterial functional profiles under stress. Additionally, in this study, we related that fungicide-affected soil physical environments. Probably, the differences in pH, clay content and organic matter concentration between the two soil types reflected differently on CH and CA bioavailability; hence, on bacterial adaptations to changed soil nutrient pools. Great differences in temporal profiles of CH and CA utilisation (not shown here) were detected in the late fungicide exposure stage (D90–D120), when the utilisation of the two CGs increased dramatically at LS (by 45%, on average) and stabilised (CH) or decreased by 15% (CA) at CL.

Higher fungicide concentrations (Az2 and Az3) widened the spectrum of impacted CGs, but these effects could be related to soil chemical pollution, rather than to the controlled use of Quadris^R for plant protection.

Metabolic diversity

In both soil types, Quadris^R application influenced metabolic evenness rather than on metabolic richness, which might be explained by the intrinsic bacterial community capacity to be metabolic resistant, resilient and redundant (Fenchel and Finlay 2004; Meyer et al. 2004). Some bacteria show a high degree of metabolic tolerance (resistance) to changing environmental conditions (Meyer et al. 2004), whereas others

are capable to adapt quickly to the new nutrient inputs for rapid growth (resilience) (Fenchel and Finlay 2004). Further, the extremely high abundance and diversity of bacteria are arguments for their metabolic redundancy, ensuring ecosystem functioning (nutrient turnover), even in extreme conditions.

We assumed that Quadris^R significantly changed soil nutrient pools and the changes might occur due to soil accumulation of dead fungal biomass, induction of detoxification agents (mainly proteins) - molecules that can later be metabolised by the same microbiota (Degens et al. 2000), changes in soil pH and/or fungicide adjuvants' inputs (Syngenta 2021). Changes in soil mesocosms affected in different ways bacterial capacity to use CSs – ranging from inhibition to stimulation. Interesting was the stimulation effects of Quadris^R on the utilisation of γ -Hydroxybutyric acid, 2-Hydroxy benzoic acid and α -Ketobutyric acid at CL. Stimulated utilisation of 2-Hydroxy benzoic acid and α -Ketobutyric acid under fungicide tetraconazole was observed earlier by Sułowicz et al. (2016).

Conclusions

The study showed that soil properties (soil texture, pH and organic and inorganic substances) were of significant importance for the fate of applied fungicide Quadris^R, as well as its effects on bacterial metabolism. The fungicide decreased for at least four months the overall bacterial activity (AWCD), shifted the metabolite profiles (CLPPs) of bacterial communities and changed the preferred carbon sources and metabolic diversity. Fungicide also affected the mode of environmental control on bacteria, in accordance with soil peculiarities.

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References

- Aleksova M (2020) Microbial assessment of soil resistance and resilience after fungicide azoxystrobin application. PhD Thesis. Sofia University “St. Kliment Ohridski”. [in Bulgarian]
- Aleksova M, Kenarova A, Boteva S, Georgieva S, Chaney Ch, Radeva G (2021) Effects of increasing concentrations of fungicide Quadris^R on bacterial functional profiling in loamy sand soil. *Archives of Microbiology* 203(7): 4385–4396. <https://doi.org/10.1007/s00203-021-02423-2>
- Baćmaga M, Kucharski J, Wyszowska J (2015) Microbial and enzymatic activity of soil contaminated with azoxystrobin. *Environmental Monitoring and Assessment* 187(10): e615. <https://doi.org/10.1007/s10661-015-4827-5>

- Baćmaga M, Wyszkowska J, Kucharski J (2017) Bioaugmentation of soil contaminated with azoxystrobin. *Water, Air, and Soil Pollution* 228(1): e19. <https://doi.org/10.1007/s11270-016-3200-9>
- Bartlett DW, Clough JM, Godwin JR, Hall AA, Hamer M, Parr-Dobrzanski B (2002) The strobilurin fungicides. *Pest Management Science* 58(7): 649–662. <https://doi.org/10.1002/ps.520>
- Bending GD, Rodríguez-Cruz MS, Lincoln SD (2007) Fungicide impacts on microbial communities in soils with contrasting management histories. *Chemosphere* 69(1): 82–88. <https://doi.org/10.1016/j.chemosphere.2007.04.042>
- Cycoń M, Wójcik M, Piotrowska-Seget Z (2011) Biodegradation kinetics of the benzimidazole fungicide thiophanate-methyl by bacteria isolated from loamy sand soil. *Biodegradation* 22(3): 573–583. <https://doi.org/10.1007/s10532-010-9430-4>
- Degens BP, Schipper LA, Sparling GP, Vojvodic-Vukovic M (2000) Decreases in organic C reserves in soils can reduce the catabolic diversity of soil microbial communities. *Soil Biology & Biochemistry* 32(2): 189–196. [https://doi.org/10.1016/S0038-0717\(99\)00141-8](https://doi.org/10.1016/S0038-0717(99)00141-8)
- Devare M, Londoño-R LM, Thies JE (2007) Neither transgenic Bt maize (MON863) nor tefluthrin insecticide adversely affect soil microbial activity or biomass: A 3-year field analysis. *Soil Biology & Biochemistry* 39(8): 2038–2047. <https://doi.org/10.1016/j.soilbio.2007.03.004>
- Edwards CA, Abe T, Striganova BR (1995) *Structure and Function of Soil Communities*. Kyoto University Press, Kyoto, 152 pp.
- Fenchel T, Finlay BJ (2004) The ubiquity of small species: Patterns of local and global diversity. *Bioscience* 54(8): 777–784. [https://doi.org/10.1641/0006-3568\(2004\)054\[0777:TUOS SP\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2004)054[0777:TUOS SP]2.0.CO;2)
- FOCUS group (2006) Guidance document on estimating persistence and degradation kinetics from environmental fate studies on pesticides in EU registration. Report of the FOCUS work group on degradation kinetics. EC Document Reference Sanco/10058/2005 version, 2.0., 434 pp.
- Garland JL (1996) Analytical approaches to the characterization of samples of microbial communities using patterns of potential C source utilization. *Soil Biology & Biochemistry* 28(2): 213–221. [https://doi.org/10.1016/0038-0717\(95\)00112-3](https://doi.org/10.1016/0038-0717(95)00112-3)
- Garland JL, Mills AL (1991) Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole carbon-sources utilization. *Applied and Environmental Microbiology* 57(8): 2351–2359. <https://doi.org/10.1128/aem.57.8.2351-2359.1991>
- Ghosh RK, Singh N (2009) Effect of organic manure on sorption and degradation of azoxystrobin in soil. *Journal of Agricultural and Food Chemistry* 57(2): 632–636. <https://doi.org/10.1021/jf802716f>
- Hammer Ø, Harper DAT, Ryan PD (2001) PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 4(1): 1–9. https://palaeo-electronica.org/2001_1/past/past.pdf
- Han Y, Zhu L, Wang J, Wang J, Xie H, Zhang S (2014) Integrated assessment of oxidative stress and DNA damage in earthworms (*Eisenia fetida*) exposed to azoxystrobin. *Ecotoxicology and Environmental Safety* 107: 214–219. <https://doi.org/10.1016/j.ecoenv.2014.06.006>

- Howell CC, Semple KT, Bending GD (2014) Isolation and characterization of azoxystrobin degrading bacteria from soil. *Chemosphere* 95: 370–378. <https://doi.org/10.1016/j.chemosphere.2013.09.048>
- ISO 11277 (2009) Soil quality—determination of particle size distribution in mineral soil material. http://www.iso.org/iso/catalogue_detail.htm?csnumber=54151
- Keeney DR, Nelson DW (1982) Nitrogen-inorganic forms. 2nd ed. In: Page AL, Miller RH, Keeney D (Eds) *Methods of Soil Analysis, Part 2, Agronomy Monograph, 9*. ASA and SSSA, Madison, 643–698. <https://doi.org/10.2134/agronmonogr9.2.2ed.c33>
- Kenarova A, Radeva G, Traykov I, Boteva S (2014) Community level physiological profiles of bacterial communities inhabiting uranium mining impacted sites. *Ecotoxicology and Environmental Safety* 100: 226–232. <https://doi.org/10.1016/j.ecoenv.2013.11.012>
- McGrath MT (2004) What are Fungicides. *The Plant Health Instructor*. [Updated 2016] <https://doi.org/10.1094/PHI-I-2004-0825-01>
- Meyer AF, Lipson DA, Martin AP, Schadt CW, Schmidt SK (2004) Molecular and metabolic characterization of cold-tolerant alpine soil *Pseudomonas* sensu stricto. *Applied and Environmental Microbiology* 70(1): 483–489. <https://doi.org/10.1128/AEM.70.1.483-489.2004>
- Mijangos I, Becerril JM, Albizu I, Epelde L, Garbisu C (2009) Effects of glyphosate on rhizosphere soil microbial communities under two different plant composition by cultivation-dependent and -independent methodologies. *Soil Biology & Biochemistry* 41(3): 505–513. <https://doi.org/10.1016/j.soilbio.2008.12.009>
- Muñoz-Leoz B, Ruiz-Romera E, Antigüedad I, Garbisu C (2011) Tebuconazole application decreases soil biomass and activity. *Soil Biology & Biochemistry* 43(10): 2176–2183. <https://doi.org/10.1016/j.soilbio.2011.07.001>
- Olsen SR (1982) Phosphorus. 2nd ed. In: Page AL, Miller RH, Keeney D (Eds) *Methods of soil analysis, Part 2, Agronomy Monograph, 9*. ASA and SSSA, Madison, 1040–1042.
- Rodrigues ET, Lopes I, Pardal MA (2013) Occurrence, fate and effects of azoxystrobin in aquatic ecosystems: A review. *Environment International* 53: 18–28. <https://doi.org/10.1016/j.envint.2012.12.005>
- Singh N, Singh SB, Mukerjee I, Gupta S, Gajbhiye VT, Sharma PK, Goel M, Dureja P (2010) Metabolism of 14C-azoxystrobin in water at different pH. *Journal of Environmental Science and Health. Part. B, Pesticides, Food Contaminants, and Agricultural Wastes* 45(2): 123–127. <https://doi.org/10.1080/03601230903471910>
- SSDS (1993) *Soil Survey Manual*. United States Department of Agriculture, 63–65. [Retrieved 30 August 2014]
- Sułowicz S, Cycoń M, Piotrowska-Seget Z (2016) Non-target impact of fungicide tetraconazole on microbial communities in soils with different agricultural management. *Ecotoxicology (London, England)* 25(6): 1047–1060. <https://doi.org/10.1007/s10646-016-1661-7>
- Syngenta (2021) QUADRIS Safety data sheet. https://www.syngenta.co.uk/sites/g/files/zhg151/f/media/2021/10/11/quadrisk_v22.0_0.pdf?token=1633945964
- Vincelli P (2002) Q₁I (Strobilurin) Fungicides: Benefits and Risks. *The Plant Health Instructor*. <https://www.apsnet.org/edcenter/disimpactmngmnt/topc/Pages/StrobilurinFungicides.aspx>

- Wang X, Lu Zh, Miller H, Liu J, Hou Zh, Liang Sh, Zhao X, Zhang H, Borch Th (2020) Fungicide azoxystrobin induced changes on the soil microbiome. *Applied Soil Ecology* 145: e103343. <https://doi.org/10.1016/j.apsoil.2019.08.005>
- Weber KP, Legge RL (2009) One-dimensional metric for tracking bacterial community divergence using sole carbon source utilization patterns. *Journal of Microbiological Methods* 79(1): 55–61. <https://doi.org/10.1016/j.mimet.2009.07.020>
- Wu X, Xu J, Dong F, Liu X, Zheng Y-Q (2014) Responses of soil microbial community to different concentrations of fomesafen. *Journal of Hazardous Materials* 273: 155–164. <https://doi.org/10.1016/j.jhazmat.2014.03.041>
- Yu B, Chen Z, Lu X, Huang Y, Zhou Y, Zhang Q, Wang D, Li J (2020) Effects on soil microbial community after exposure to neonicotinoid insecticides thiamethoxam and dinotefuran. *The Science of the Total Environment* 725: e138328. <https://doi.org/10.1016/j.scitotenv.2020.138328>
- Zhang W, Xu J, Dong F, Liu X, Zhang Y, Wu X, Zheng Y (2014) Effect of tetraconazole application on the soil microbial community. *Environmental Science and Pollution Research International* 21(13): 8323–8332. <https://doi.org/10.1007/s11356-014-2844-5>