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The allelopathic properties of decomposing buckwheat residues are not directly related to phenolic compounds in soil

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Abstract: Previous studies have shown that residues of common buckwheat roots (BRR) (but not entire common buckwheat plants, BPR) in the soil inhibited the growth of barnyardgrass (*Echinochloa crus-galli* L.). The objective of the study was to determine how both the residues affect the content of free phenolics, their esters and glycosides in the soil. The aqueous extracts were used to analyse of unbound phenolic compounds, while those bound to the soil were extracted with sodium citrate. Moreover, an *in vitro* test was used to assess the allelopathic effect of phenolic compounds present in the soil against barnyardgrass. Among the analysed phenolic compounds after 7 days of BPR and BRR decomposition, only *ortho-*, *meta-* and *para*-coumaric acids and apigenin were found in measurable amounts in the soil. The concentrations of free phenolic compounds were very low. Much higher contents occurred for the esters of these compounds, while no glycosides were found. The contents of phenolic compounds bound to soil were many times higher than unbound ones. The 37-day decomposition period resulted in an increase in bound phenolics, while the content of unbound changed slightly. Overall, the levels of phenolic compounds in the soil with the BRR-amended soil and no-buckwheat residue control were low, and significantly higher in the soil with BPR. An *in vitro* test showed that *m-*, *p*-coumaric acids and apigenin added to growth medium at a concentration higher than in the soil did not affect barnyardgrass shoot growth. Since the levels of phenolic compounds in the soil containing BRR and control soil were low and similar, phenolic compounds cannot be directly responsible for the allelopathic properties caused by the presence of BRR.

Keywords: *Fagopyrum esculentum* Moench; allelopathy; *Echinochloa crus-galli* L.; phenylpropanoid; competition; growth inhibitor

Plants may compete with one another for basic resources: mineral nutrients, water and light. They may also produce allelochemicals that directly or indirectly affect the growth of neighboring plants (Cheng and Cheng 2015). Allelochemicals released by plants and/or decaying plant residues can regulate the soil microbial community and chemical and physical properties of the soil, which may indirectly inhibit the growth of competing plant species (Javaid 2007).

Phenolics are major allelochemicals in the soil ecosystem and they play important role in allelopathy (John and Sarada 2012). These compounds can be released into the soil from the living plants or as a result of the decomposition of plant residues or as microbial by-products of plant residue decomposition. They can interact with cell membranes, alter ion fluxes, hydraulic conductivity of roots, and nutrient uptake (Einhellig 2004). Blum and Gerig (2005) concluded that the level of individual phenolic compounds is insufficient to inhibit plant growth, but rather their mixture or composition with other compounds.

The concentration of phenolics in soil decreased due to their instability, and it has a great impact on their potential allelopathic properties (Li et al. 2015).

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Various environmental factors, such as soil pH, temperature, and oxygen, can affect the degradation of phenolics. Phenolic acids may be rapidly sorbed by soil particles. In the soil solution, the concentration of phenolic acids bound to soil was several times higher than that of free ones (Fujii et al. 1991). As soon as 8 h after their addition, more than 80% of the acids are bound to the soil (Cecchi et al. 2004). It has been shown that the allelopathic properties of these compounds can be suppressed by the sorption process which depends on their molecular size and polarity (Seigler 1996). Water is use for extraction of the free phenolics in the soil, while citrate solution is applied for extraction of the phenolics reversibly bound to the soil particles (Blum 1997).

Activity of allelochemicals depends on the time after their release to soil. The greatest phytotoxic activity was noticed at an early stage of plant residues decomposition (Kitou 1999, Ismail and Chong 2002). Smeda and Weller (1996) have demonstrated that the allelopathic effect of plant residues on weeds declines after 4–6 weeks due to breakdown of allelochemicals. The concentration of phenolic acids in soil increased during the first 3–7 days after rye termination and then decreased to the initial level after 56 days (Otte et al. 2020). The role of microorganisms in the changes of allelopathic effects has also been extensively studied and the results demonstrated their importance in terms of activation or degradation of allelochemicals (Zhang et al. 2010). Although crop residues have great weed control potential, there have been reports that they may also affect cultivated plants. Pużyńska et al. (2019) demonstrated that common buckwheat seed-meal removed barnyardgrass *(Echinochloa crus-galli* L.*)* from the open field plots but also inhibited maize growth.

It is well known that common buckwheat (*Fagopyrum esculentum* Moench) has substantial allelopathic properties (Kalinova et al. 2007, Golisz et al. 2007, Pużyńska et al. 2019, Szwed et al. 2019b). Studies on the allelopathic properties are often carried out using various extracts from plants for weed germination and/or the growth (Golisz et al. 2007). An aqueous extract from a 14-day plants of common buckwheat inhibited seedling growth of several weed species, including barnyardgrass (Szwed et al. 2019a). It is believed that phenolic acids and flavonoids are mainly responsible for buckwheat allelopathic properties (Iqbal et al. 2003, Kalinova et al. 2005, Golisz et al. 2007, Pużyńska et al. 2019). Among buckwheat phenolic compounds, catechin had the highest allelopathic activity but, due to the low level, its potential as a growth inhibitor could be important as a root exudate (Kalinova and Vrchotová 2009).

Also, buckwheat residues in the soil suppressed the growth of several weed species (Kumar et al. 2008, 2009). However, in some cases, the allelopathic characteristics of buckwheat and its compounds are difficult to explain (Wirth and Gfeller 2016). The authors investigated the suppression of pigweed growth by buckwheat exudates, and were unable to prove that the observed effect was at least partialy caused by allelopathic compounds occurring there. This was explained by the fact that allelochemicals were present at a very low concentrations.

It has recently been found that common buckwheat root residues (BRR) in soil have higher allelopathic potential than residues of entitre buckwheat plant (BPR) (Szwed et al. 2019b). The biomass of 30-day-old plants of barnyardgrass and cleavers grown in bare soil was approximately 5- and 3.5-fold higher, respectively, than in plants grown in the presence of BRR. However, BPR incorporated into the soil insignificantly affected the biomass of both weed species studied. Therefore, the objective of the study presented here was to show whether the greater allelopathic properties of buckwheat root residues in the soil is related to the content of phenolic compounds therein. The main aim of the study was compare the impact of buckwheat residues (BRR, BPR) on the contents of phenolic compounds derivatives (free, esters, and glycosides), unbound and bound to the soil.

MATERIAL AND METHODS

Common buckwheat (*Fagopyrum esculentum* Moench, cv. Hruszowska) seeds were sown in four containers filled with commercial horticultural soil (6 kg; HIT-TORF, pH 6.48; 6.9 g N/kg; 0.65 g P/kg, Poland). Temperature in the air-conditioned plant growth room was maintained at 24 ± 2 °C during the day (16 h) and 18 ± 2 °C during the night (8 h). Photosynthetically active radiation (PAR) (ca. 100 µmol/m^2/s) was provided by high-pressure sodium lamps (400 W, Plantaster, Osram, Germany). The containers with growing buckwheat were watered every morning. After 14 days of buckwheat plant growth, the above-ground parts of buckwheat plants were removed, but the roots remained (BRR) in the soil for additional 7 days. The mean initial biomass of BRR in two containers was 32.2 g/kg of soil. Whole buckwheat plants (BPR) were incorporated into the soil in the other two

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containers after they had been cut into small parts. The mean biomass concentration of BPR was 86.8 g/kg of soil. Tiny vetch seeds were sown (100–120 seeds per one container) in containers containing BPR and BRR residues and in control containers containing bare soil, and allowed to grow for 30 days.

Extraction of phenolic compounds from soil. Initially, soil containing buckwheat residues was sampled for analysis 7 days after the start of the decomposition process. Extraction of phenolic compounds was carried out using water or sodium citrate solution. The extracts was made from 200 g of soil which was ground in a mortar and shaken with 400 mL of distilled water or 0.25 mol/L sodium citrate (pH = 7.0) for 2.5 h (Blum 1997). The obtained extracts were centrifuged (3 500 rpm) for 20 min, and then filtered through Whatman 4 filter paper. Next, the extracts were freeze-dried. After sowing tiny vetch seeds into three types of soil (containing buckwheat root residues, residues of entire buckwheat plants or soil without residues) and 30 days of growth, a similar extraction procedure was carried out.

Determination of free and bound phenolic acids and flavonoids. Phenolic compounds (phenolic acids, flavonoids) were analysed by HPLC-MS/MS for the concentration of their various derivatives (free, esters, and glycosides). The profile and content of phenolic compounds were determined according to the method described previously (Szwed et al. 2019b). Briefly, the phenolic derivatives extracted were dissolved in 80% methanol and subjected to HPLC-MS/MS analysis. Aliquots of extracts were injected into an HPLC system equipped with an HALO C_{18} column (2.7 µm particles, 0.5 × 50 mm, Eksigent, Vaughan, Canada). For HPLC-MS/MS analysis, a QTRAP 5500 ion trap mass spectrometer (AB SCIEX, Vaughan, Canada) was used. Qualitative and quantitative analyses were conducted in the negative mode by multiple reaction monitoring of selected ions. The following phenolic compounds (free, esters and glycosides) were analysed: *trans*-cinnamic acid, ferulic acid, *o*-, *m*-, *p*-coumaric acids, chlorogenic acid, caffeic acid, sinapic acid, vanillic and *iso*-vanillic acid, protocatechuic acid, syringic acid, catechin, luteolin, apigenin, kaempferol, and quercetin.

Bioassays involving free phenolic compounds found in soil. Assessment of the influence of *o*-, *m*-, *p*-coumaric acids and apigenin on the growth of barnyardgrass seedlings was carried out for the concentrations 0.0016–16.4 µg/mL for coumaric acids, and 0.0024–24.0 µg/mL for apigenin. Initially, barnyardgrass seeds were germinated between two strips of rolled wet paper placed vertically in a beaker containing 1/5 of Hoagland's solution. Germination lasted for 4 days in the dark at 24 ± 1 °C. The etiolated seedlings were then transferred to a growth room where they were kept for 3 days and grown in conditions as described above. After measuring the shoot and main root length in the 7-day old barnyardgrass seedlings, they were then placed in beakers containing 200 mL of solution of the tested compound or water (control). Due to the instability of phenolic compounds in water solution (Roger et al. 1999, Li et al. 2015), after 2 days of the experiment, the plants were transferred to beakers containing freshly prepared solutions of the compounds. An increase in root and shoot length (elongation) of barnyardgrass seedlings was determined after 2 and 5 days of exposure to solution of phenolic compounds.

RESULTS

In extracts obtained from soil, the following phenolic compounds were analysed: *trans*-cinnamic acid, ferulic acid, *o*-, *m*-, *p*-coumaric acids, chlorogenic acid, caffeic acid, sinapic acid, vanillic and *iso*-vanillic acid, protocatechuic acid, syringic acid, catechin, luteolin, apigenin, kaempferol, and quercetin. Of these, measureable amounts of *ortho*-, *meta*- and *para*-coumaric acids and apigenin (Table 1) were found in soil extracts after 7 days of decomposition of buckwheat residues. In the aqueous extract, *meta*-coumaric acid was the only free acid present in measurable amounts. However, its content was very low, below 0.03 µg/g of soil. Coumaric acids appeared mainly in the form of their ester derivatives, exceeding several times the level of their free forms.

Among soil-bound acids (released with sodium citrate solution), ester derivatives of coumaric acids also dominated quantitatively, exceeding many times the content of their free forms. In addition, the level of the only measurable free (*meta*-coumaric) acid was several folds higher than the content of unbound acid. The content of esters of free and soil-bound coumaric acids was significantly higher in soil with buckwheat residues than in bare soil.

After a longer, that is 37-day, decomposition of buckwheat residues in the soil, there was a slight increase in the content of free coumaric acids and caffeic acid unbound to soil (Table 2). In the case of acid esters, their content also increased. Unlike after 7 days of decomposition, after 37 days, these acids also appeared as glycosidic derivatives. The highest level of these compounds was found in BPR-

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Table 1. Phenolic compound content (μg/g of fresh soil) after seven days' decomposition of buckwheat residues in soil

Analysed compound	Soil	Water extract			Citrate extract		
		free	esters	glycosides	free	esters	glycosides
o-Coumaric acid	control	nd	0.032^{b}	nd	nd	0.051^{b}	nd
	+ BPR	nd	0.091^{a}	nd	nd	0.084^{b}	nd
	+ BRR	nd	0.101^{a}	nd	nd	0.142^{a}	nd
m-Coumaric acid	control	0.012^{a}	0.082^{b}	nd	0.461^{b}	3.111^{b}	nd
	+ BPR	0.012^{a}	0.211^{a}	nd	0.905^{a}	5.444^{a}	nd
	+ BRR	0.031^{a}	0.264^{a}	nd	0.470^{b}	3.294^{b}	nd
p-Coumaric acid	control	nd	0.042^{b}	nd	nd	0.971^{c}	nd
	+ BPR	nd	0.101^{a}	nd	nd	2.921^{a}	nd
	+ BRR	nd	0.094^{a}	nd	nd	1.863^{b}	nd
Ferulic acid	control	nd	nd	nd	nd	nd	nd
	+ BPR	nd	nd	nd	nd	nd	nd
	+ BRR	nd	nd	nd	nd	nd	nd
Caffeic acid	control	nd	nd	nd	nd	nd	nd
	+ BPR	nd	nd	nd	nd	nd	nd
	+ BRR	nd	nd	nd	nd	nd	nd
Quercetin	control	nd	nd	nd	nd	nd	nd
	+ BPR	nd	nd	nd	nd	nd	nd
	+ BRR	nd	nd	nd	nd	nd	nd
Apigenin	control	0.012^{a}	nd	nd	0.942^{ab}	nd	nd
	+ BPR	0.011^{a}	nd	nd	0.881^{b}	nd	nd
	+ BRR	0.011^{a}	nd	nd	1.134^{a}	nd	nd
Total phenolic compounds	control	0.024^{a}	0.156^{b}	nd	1.403^{ab}	4.133^{c}	nd
	+ BPR	0.023^{a}	0.402^{a}	nd	1.786^{a}	8.469^{a}	nd
	+ BRR	0.042^{a}	0.459^{a}	nd	1.604^{a}	5.309^{b}	nd

nd – means no presence or content below 0.010 μg/g of fresh soil; BPR – residues of entire buckwheat plants; BRR – buckwheat plant roots. One-way ANOVA with Tukey's test was performed. Different letters indicate significant differences between soils ($P < 0.05$)

amended soil. The differences between bare soil and soil containing BRR were small or non-existent.

Analyses of phenolic compounds bound to soil showed a relatively large increase in the content of free *o*-, *m*- and *p*-coumaric acids compared to the state after 7 days of the presence of buckwheat tissue in the soil. Among the three tested soils, the highest content of these compounds occurred in BPR-amended soil, it being similar in bare soil and BRR-amended soil. A similar situation was in the case of ester derivatives of these acids, except that their contents were many times higher than the levels of free acids. The levels were also much higher compared with 7-day decomposition (Tables 1 and 2).

Comparison of these three soils in terms of the content of these compounds in most cases followed the pattern determined for free acids and their esters: the highest amounts occurred in soil with the addition of BPR while in BRR-amended soil and bare soil these contents were similar.

An *in vitro* experiment showed that three isomers of coumaric acid and apigenin added to growth medium in concentrations of up to 16.4 μg/mL insignificantly affected shoot growth of barnyardgrass seedlings after 2 and 5 days of exposure (Table 3). Of the acids, only the concentration of 16.4 μg/mL of *o*-coumaric acid inhibited the growth of the barnyardgrass seedling primary root. However, the same concentration of *p*-coumaric acid stimulated the growth of the root after 2 days of exposure. Higher apigenin concentrations (0.24 and 24.0 μg/mL) also stimulated the growth of the primary root after 5-day exposition of barnyardgrass seedlings.

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Table 2. Phenolic compound content (μg/g of fresh soil) after 37 days' decomposition of buckwheat residues in soil

Analysed compound	Soil	Water extract			Citrate extract		
		free	esters	glycosides	free	esters	glycosides
o-Coumaric acid	control	nd	0.042^{b}	nd	0.182^{b}	7.212^{b}	0.353^{a}
	+ BPR	nd	0.101^{a}	0.012^{a}	0.421^{a}	8.111^{a}	0.452^{a}
	+ BRR	nd	0.032^{b}	0.011^{a}	0.262^{b}	7.312^{b}	0.131^{b}
m-Coumaric acid	control	0.032^{a}	0.181^{a}	0.022^{a}	1.551^{b}	19.23^{b}	2.040^{b}
	+ BPR	0.051^{a}	0.262^{a}	0.032^{a}	4.843^{a}	40.62^{a}	4.951^{a}
	+ BRR	0.043^{a}	0.093^{b}	0.021^{a}	1.922^{b}	18.28^{b}	1.532^{b}
p-Coumaric acid	control	nd	0.042^{b}	nd	0.601^{b}	8.521^{b}	0.541^{b}
	+ BPR	nd	0.101^{a}	0.012^{a}	1.023^{a}	10.25^{a}	0.961^{a}
	+ BRR	0.011	0.091^{a}	0.013^{a}	0.711^{b}	9.082^{b}	0.512^{b}
Ferulic acid	control	nd	nd	0.012^{a}	0.230^{a}	1.862^{a}	1.411^{a}
	+ BPR	nd	nd	0.011^{a}	0.190^{a}	2.101^{a}	1.691^{a}
	+ BRR	nd	nd	0.012^{a}	0.181^{a}	1.712^{a}	1.562^{a}
Caffeic acid	control	nd	0.052^{b}	0.043^{a}	0.262^{b}	11.20^{b}	9.331^{a}
	+ BPR	nd	0.101^{a}	0.071^{a}	0.411^{a}	14.76^{a}	9.400^{a}
	+ BRR	nd	0.051^{b}	0.042^{a}	0.180^{c}	10.78^{b}	8.000^{b}
Quercetin	control	nd	nd	nd	nd	nd	0.441^{a}
	+ BPR	nd	nd	nd	nd	0.381^{a}	0.370^{a}
	+ BRR	nd	nd	nd	nd	0.481^{a}	0.502^{a}
Apigenin	control	nd	nd	nd	nd	nd	0.161^{c}
	+ BPR	nd	nd	nd	nd	nd	0.771^{a}
	+ BRR	nd	nd	nd	nd	nd	0.330^{b}
Total phenolic compounds	control	0.032^{a}	0.317^{b}	0.077^{b}	2.826^{b}	48.03^{b}	14.28^{b}
	+ BPR	0.051^{a}	0.565^{a}	0.138^{a}	9.708^{a}	76.22^{a}	18.60^{a}
	+ BRR	0.054^{a}	0.267^{b}	0.099^{b}	3.256^{b}	47.64^{b}	12.58^{c}

nd – means no presence or content below 0.010 μg/g of fresh soil; BPR – residues of entire buckwheat plants; BRR – buckwheat plant roots. One-way ANOVA with Tukey's test was performed. Different letters indicate significant differences between soils ($P < 0.05$)

DISCUSSION

It has recently been found that common buckwheat root residues in soil have higher allelopathic potential than the entitre buckwheat plant residues (Szwed et al. 2019b). The biomass of 30-day-old plants of barnyardgrass grown in bare soil was approximately 5-fold higher than in plants grown in the presence of BRR. However, the presence of BPR in the soil did not significantly affect the biomass of this weed. So far, phenolic compounds have been assumed to be responsible for the allelopathic properties of common buckwheat (Iqbal et al. 2003, Kalinova et al. 2005, Golisz et al. 2007, Kalinova and Vrchotova 2009). It has therefore been hypothesised that different composition and/or concentration of phenolic compounds may be responsible for differences between the allelopathic activity of BRR and BPR.

Among the 12 phenolic acids and 5 flavonoids analysed, the following were found to be present: *ortho-*, *meta-* and *para*-cumaric acids, caffeic and ferulic acids, and also quercetin and apigenin glycosides. The results of soil extract analyses show that the content of phenolics unbound to soil particles is very low as they represent only a few percent of the total amount of these compounds. These data confirm previous reports (Fujii et al. 1991, Seigler 1996, Cecchi et al. 2004). According to Cecchi et al. (2004), as soon as 8 h after their addition, more than 80% of the phenolic acids were bound to the soil. Also, Ohno et al. (2000) have found, for decomposed clover residue, low levels of phenolics due to their sorption or oxidiation. Phenolic compounds, both unbound and bound with soil, occur predominantly as ester derivatives. These proportions changed insignificantly after the prolonged time of decomposition of buckwheat residues. So far,

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Table 3. The effect of 2-day and 5-day exposure to phenolic compounds added to the growth medium on elongation (mm) of the shoot and primary root of barnyard grass seedlings

	Concentration in growth medium (acid/apigenin, μg/mL)	*p*-Coumaric acid		*o*-Coumaric acid		*m*-Coumaric acid		Apigenin	
		2 days	5 days	2 days	5 days	2 days	5 days	2 days	5 days
Primary root	control (water)	25.92^{b}	55.71^{a}	18.90^{a}	56.41^{a}	26.12^{a}	45.91^{a}	9.14^{a}	25.51^{c}
	0.0016/0.0024	25.81^{b}	58.12^{a}	17.92^{a}	55.72^{a}	24.40^{a}	48.23^{a}	10.11^{a}	34.92^{b}
	0.016/0.024	26.32^{b}	55.42^{a}	18.53^{a}	59.32^{a}	28.61^{a}	47.11^{a}	8.18^{a}	26.20^{c}
	0.16/0.24	31.01^{b}	59.55^{a}	19.52^{a}	60.14^{a}	24.23^{a}	43.14^{a}	10.72^{a}	40.63^{a}
	16.4/20.4	35.64^{a}	59.53^{a}	11.92^{b}	20.33^{b}	28.33^{a}	45.12^{a}	11.22^{a}	43.24^{a}
Shoot	control (water)	11.51^{a}	15.74^{a}	14.04^{a}	22.04^{a}	9.96^{a}	24.04^{a}	10.85^{a}	17.34^{a}
	0.0016/0.0024	11.83^{a}	16.11^{a}	13.71^{a}	22.21^{a}	9.17^{a}	26.21^{a}	11.63^{a}	18.24^{a}
	0.016/0.024	12.33^{a}	16.00^{a}	14.03^{a}	22.03^{a}	10.02^{a}	26.03^{a}	10.72^{a}	19.32^{a}
	0.16/0.24	11.82^{a}	15.72^{a}	15.42^{a}	22.22^{a}	9.55^{a}	25.13^{a}	11.91^{a}	17.02^{a}
	16.4/20.4	12.34^{a}	16.93^{a}	13.36^{a}	22.76^{a}	9.83^{a}	26.06^{a}	12.34^{a}	18.45^{a}

One-way ANOVA with Tukey's test was performed. Different letters indicate significant differences between doses of phenolics ($P < 0.05$)

composition of free, esters and glycosides in soil has not been determined for buckwheat residues.

An *in vitro* experiment showed that coumaric acids and apigenin added to growth medium in concentrations of up to 16.4 μg/mL insignificantly affected shoot growth of barnyard grass seedlings after 2 and 5 days of exposure. Of the acids, only the concentration of 16.4 μg/mL of *o*-coumaric acid inhibited the growth of the primary root of barnyardgrass. However, the same concentration of *p*-coumaric acid stimulated the growth of the root after 2 days of exposure. Higher apigenin concentrations (0.24 and 24.0 μg/mL) also stimulated the growth of the primary root after 5 days of the presence of this flavonoid in the growth medium. Experiments on the effect of phenolic acids applied to growth medium have indicated that their concentrations in soil are too low to have an allelopathic effect on barnyardgrass.

Comparisons of the content of free phenolic compounds in the soil clearly demonstrate that their low level cannot be directly responsible for inhibition of barnyardgrass growth.

Previously, Wirth and Gfeller (2016) stated that the allelopathic characteristics of buckwheat and its compounds are difficult to explain. Although buckwheat root exudates suppressed the growth of the pigweed, this effect could not be caused by the compounds present, as they were present at very low concentrations. It seems that the results of the work reported here confirm these observations.

Due to complexity of the soil environment, it is very difficult to demonstrate that concentrations of phenolic compounds found in soils function as allelopathic agents that inhibit or stimulate plants growing there (Walker et al. 2003). The contents of individual phenolic compounds in soil with and without BRR (control) were generally the same or similar. This is an important finding confirming the fact that their direct role in allelopathy is minor or even negligible. Moreover, the enhanced content of phenolic compounds in the soil containing BPR was not accompanied by inhibition of barnyardgrass growth, as was the case in the BRR-amended soil (Szwed et al. 2019b). The content of soil-bound phenolic compounds which was many times higher compared with their free counterparts may highlight other problems that need to be clarified: are these compounds important in allelopathy and are organisms living in the soil (plants, bacteria, fungi) able to desorb these compounds?

The content of phenols in the soil is known to decrease due to their instability caused by chemical processes in the soil and metabolic processes in microorganisms (Zhang et al. 2010, Li et al. 2015). Decaying plant residues can regulate the soil microbial community as well as chemical and physical properties of the soil, which may indirectly affect the growth of weeds (Javaid 2007). This may be another important factor affecting plants growing in such soil that needs to be examined.

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