Allelochemicals-based expansion of Elsholtzia densa Benth. hindering plants'defense function: case study of Hordeum vulgare L.

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Abstract

Background The root extracellular trap (RET) comprising root border cells (RBCs) and their secretions are plants' first line of defense to resist external stress. Since soil microorganisms are closely related to nutrient circulation, this study chooses a poisonous weed *Elsholtzia densa* Benth. (*E. densa*) in alpine meadows on the Qinghai-Tibet Plateau (QTP) to explore the mechanism of its rapid expansion.

Methods Bioassays and pure agar suspension air culture methods are used to determine the effects of the decomposing substances of *E. densa* on the RBCs characteristics of the plateau crop highland barley (*Hordeum vulgare* L.), soil nutrients, soil enzyme activities, and soil bacteria, using microscopic techniques and biochemical analysis techniques.

Results The decomposing allelopathic effect of the *E. densa* mainly occurs in the early stage of decomposing, with its decomposing solution thickening the RBC's mucilage layer and decreasing the RBC's activity and even apoptosis. The decomposed product of the *E. densa* changes the diversity of the soil bacterial and species composition, affects soil nutrient content, and increases the activities of various extracellular enzymes.

Conclusions During the expansion, the *E. densa* releases allelochemicals to its surroundings interfering with the surrounding plants' defense function and directly inhibiting their growth. At the same time, the stubble's decomposition changes soil microbial activities, enriches the soil nutrients, and forms a self-interested soil environment. Eventually, the *E. densa* gains an edge over the competition.

Key Message

Allelochemicals-based expansion of *Elsholtzia densa* Benth. hindering plants' defense function: case study of *Hordeum vulgare* L.

Introduction

Alpine meadow is the most critical grassland type on the Qinghai-Tibet Plateau (QTP), accounting for more than 66% of the total grassland are (Qin et al. 2015). In recent decades, the combined effect of many unfavorable factors significantly degraded the alpine meadows on the QTP (Geissler et al. 2019). Currently, poisonous weeds with stronger survival competitiveness expand (Peng et al. 2020), and gradually replace the high-quality forages of the native grasslands as new dominant species. The poisonous weeds compete with crops for natural resources such as light, water, and nutrients (Mushtaq et al. 2019) and release allelochemical inhibitors into the soil to inhibit the growth of other plants in the habitat. The latter is achieved by changing the soil properties and soil bacterial communities and indirectly interfering with the growth and development of surrounding plants, similar to the "new weapon hypothesis" of invasive plants accelerating their expansion (Qu et al. 2021).

During evolution and in response to external stresses, including allelopathic stress, root tips that were originally exposed directly to soil generated and released root border cells (RBCs) (Ropitaux et al. 2020). The RBCs and the mucus secreted by the plant themselves form a protective sheath of the root tip, which builds a biological, chemical, and physical barrier between the plant-root-external environment. The RBC can attract and capture soil organisms and microorganisms, adsorb and neutralize the soil's toxic substances and regulate the rhizosphere environment (Carreras et al. 2020). Therefore, the allelochemicals entering the soil must break through the biological defense line constructed by the RBCs to affect the growth and development of the root system.

In the microenvironment comprising plant root-soil-microbe interactions, the soil microbes are the bridge connecting plants-soil and plants-plants. They can decompose complex components in the soil to form small molecular substances, helping the plant roots to absorb nutrients and improve stress resistance (Zhang et al. 2020). However, the allelochemicals released by plants disrupt the balance between the surrounding plants and soil and establish new microbial communities mutually beneficial to themselves (Li et al. 2022). Soil bacteria have a greater impact on alpine meadow soil potential functions (nutrient cycling and climate regulation) than soil fungi (Wang et al. 2021).

Elsholtzia densa Benth. (*E. densa*) is a dominant poisonous weed widely distributed in the alpine meadows of the QTP (Duan et al. 2019). As an annual herb, *E. densa* produces a large amount of litter every year, and its decomposition inevitably releases allelochemicals into the soil. Therefore, several questions arise. Namely, how do the allelochemicals released by the *E. densa* through the putrefaction break through the defense line that interferes with the surrounding plants? How do the soil properties and bacterial communities change in the process? Clarifying these issues is crucial to explaining the competitive advantage of *E. densa* in alpine meadows. Therefore, this study uses the highland barley (*Hordeum vulgaere* L.), a widely grown crop in the QTP, as the receptor to evaluate the allelopathic potential of the decomposed solution of *E. densa* and its effects on the defense indicators of RBCs. In addition, soil chemical analysis and high-throughput sequencing technology of V3-V4 region fragments of 16S rDNA are used to explore the changes in the nutrient content, extracellular enzyme activities, and bacterial diversity in the decomposed soil of *E. densa.*

Materials And Methods

Materials and sources

The plants and soil of E. densa are collected from the alpine meadow at an altitude of 3470 m in Axi Township (102°55.944' E, 33°41.023' N), Hongyuan County, Aba Tibetan, and Qiang Autonomous Prefecture, Sichuan Province, China. The entire plant is divided into roots and aerial parts, dried in the shade under natural conditions, and cut into small sections of about 2 cm. The soil sample is the alpine meadow soil without the growth of E. densa, sieved into 1 mm. The highland barley seeds are purchased from Daofu County, Garze Tibetan Autonomous Prefecture.

Allelopathic effect and cytotoxicity experiments of decomposing solution

According to the actual biomass of E. densa, the decomposed solution is prepared according to 25 g of roots / 50 g of aerial parts of E. densa:500 g of soil: 1 L of water. The root decomposed solution is recorded as RD, and the aerial part of the decomposed solution is recorded as AD. After stirring evenly, both are decomposed for 30 d and 60 d, respectively, after which the mixture is filtered and made up to 1 L to obtain raw RD (25 mg/mL) and raw AD (50 mg/mL). Each treatment is repeated three times. During the experimental treatment, both are diluted with distilled water to obtain different concentrations of treatment solutions. The RD concentrations are 5, 10, 15, 20 and 25 mg/mL, and the AD concentrations are 10, 20, 30, 40 and 50 mg/mL.

The highland barley seeds with full grains and no pest damage are sterilized with 0.5% KMnO4 solution for 15 min and then rinsed repeatedly with distilled water. After soaking in a 25°C incubator for 4 h, the seeds are spread evenly in a germination box (12 cm × 12 cm × 9 cm) padded with two layers of filter paper, with 60 seeds per box. 15 mL of different concentrations of RD and AD are added to the germination box, and 15 mL of distilled water is used as the control. The number of germinated seeds and rooting is counted every day in a 25°C incubator for 7 d. The germinated germ reached half the length of the seed as germination, and the radicle reached half the length of the seed as rooted. Next, we calculate the following parameters (Sun et al. 2021):

$$GR = \frac{GN}{SN} \times 100\%$$

$$GI = \frac{G_t}{D_t}$$

$$RR = \frac{RN}{SN} \times 100\%$$

where GR is the germination rate, GN is the number of germinated seeds, SN is the total number of seeds, GI is the germination index, Gt is the germination number on day t, Dt is the corresponding germination day, RR is the rooting rate, and RN is the number of rooted seeds.

The sterilized seeds are soaked in a 25°C incubator for 12 h and then transferred to a porcelain plate covered with moist double-layer sterile gauze for 24 h without light. After the seeds are exposed to the white root tips, the full seeds are selected and inserted into a small pot covered with 3 cm perlite (diameter 10 cm, height 6 cm, 300 g perlite). After the first true leaves of the highland barley seedlings grow, 20 mL of different concentrations of RD and AD are added, distilled water is used as control, and each treatment is repeated three times. After 7 d of treatment, the roots are washed with distilled water and dried with filter paper, and the plant height and root length of the highland barley seedlings are measured. Then the dry weight of the seedlings and roots are measured (Sun et al. 2021).

The radicles of the highland barley seeds with exposed white root tips are inserted into a culture flask (volume 200 mL, diameter 9 cm, height 8.5 cm) containing 0.8% pure agar medium and cultured upside down. When the root length reaches 40 mm (at this time, the number of RBCs reaches the peak and the activity is high), 10 root tips with a length of about 5 mm are randomly selected and placed in 100 μL of

25 mg/mL RD and 50 mg/mL AD treatment solutions vortexed for 30 s. Then we rinse the root tip twice with 50 μL of treatment solution to prepare a cell suspension (200 μL), which is mixed and placed in an incubator at 25°C for 30 min in the dark, and each treatment is repeated six times.

Referring to Singh's method (Singh et al. 2021), 20 μL of cell suspension is added to a centrifuge tube (0.5 mL capacity), 8 μL of AO/EB mixed dye solution is added dropwise and stained for 2 min. The cell viability and morphology are observed under a LEICA DM300 fluorescence microscope, and each treatment is repeated five times. Then we calculate the cell viability:

Referring to the method of Ropitaux (Ropitaux et al. 2020), 8 μL of the cell suspension is taken, and 8 μL of India ink is added. After being stained for 10 min, the cells are observed with a Nikon ECLIPSE 55i microscope at x40 in a bright field, and pictures are captured to measure the thickness of the mucilage layer. Each treatment is repeated six times.

Determination of soil properties

We pulverize the dried plants of E. densa and prepare decomposed soil according to the residue content of E. densa in the soil of 0, 0.02, 0.04 g/g, and 0.08 g/g and add an appropriate amount of distilled water to keep the soil moist. Each treatment is repeated three times, and the soil samples are collected on 20 d, 40 d, and 60 d, respectively, some of which are tested for soil nutrients and enzyme activities, while the others are stored in a -80℃ refrigerator to determine bacterial diversity.

References for soil nutrient determination methods with slight modifications (Pan et al. 2016, Geng et al. 2017, Li et al. 2021). The soil's pH is measured based on the electrode potential method (air-dried soil: distilled water = 1:2.5). The soil organic matter (SOM) is determined by the potassium dichromate volumetric method, while the total phosphorus (TP) and the available phosphorus (AP) are determined using the sodium hydroxide alkali fusion-molybdenum antimony anti-colorimetric method. The absorbance values at 585 nm, 700 nm, and 882 nm are measured using a SpectraMax M2 microplate reader (Molecular Devices, USA). Both the total potassium (TK) and available potassium (AK) are measured using a Z-2000 Zeeman atomic absorption spectrophotometer and the ammonium acetate extraction-flame photometer method. The total nitrogen (TN) and available nitrogen (AN) are determined by an automatic Kjeldahl analyzer (FOSS, KJELTECTM 2300).

References determination of soil enzyme activity (Xie et al. 2020, Chen et al. 2020). The urease activity is determined by the sodium phenolate colorimetric method, and the invertase and cellulase activities are determined by the 3,5-dinitrosalicylic acid colorimetric method. The polyphenol oxidase activity is determined by the colorimetric method for measuring pyrogallol, while the acid phosphatase activity is measured using the Solarbio soil acid phosphatase (S-ACP) activity assay kit.

The genomic DNA of the soil samples is extracted using the DNA extraction kit to detect the DNA concentration and purity. By employing the diluted genomic DNA as a template, the V3-V4 region of the

bacterial 16S DNA is amplified by PCR using specific primers with Barcode and the Takara Ex Taq high-fidelity enzyme (Takara, Japan). The primer sequences are the 343 F (5'-TACGGRAGGCAGCAG-3') and 798 R (5'-AGGGTATCTAATCCT-3'). After performing electrophoresis detection on the PCR product and purifying it using magnetic beads as a template for two rounds of PCR amplification, the second-round PCR products are electrophoresed again and purified with magnetic beads for Qubit quantification. An equal number of samples are mixed according to the PCR product concentration, and the high-throughput sequencing analysis of the soil bacteria 16S rDNA is performed using the Illumina platform Miseq high-throughput sequencing technology.

Statistical analysis

According to the results of various bioassay indicators, we calculate the allelopathic response index (RI) (Williamson et al. 1988) as follows:

$$RI = 1 - \frac{C}{T} (T \geq C)$$

$$RI = \frac{T}{C} - 1 (T < C)$$

where T is the treatment value, and C is the control value. RI > 0 refers to promotion, and RI < 0 to inhibition, where the absolute value is proportional to the intensity of the allelopathy. The integrated allelopathic sensitivity index formula (SI) is the arithmetic mean of RI.

The one-way analysis of variance (ANOVA), Tukey's test for multiple comparisons (LSD), and Pearson's correlation analysis are performed using the SPSS software (IBM, SPSS Version 20.0), with a confidence level of 95%. The redundancy analysis (Canoco 5.0) studies the correlation between soil factors and dominant bacterial phyla. The bioinformatics analysis uses the Vsearch (version 2.4.2) software to classify the high-quality sequence valid tags obtained from quality control according to 97% similarity to out and selected the most abundant sequence in each OTU as the representative OUT sequence. The Chao1 index (S) size estimates the total number of soil bacterial species. Finally, the graphical representation uses Excel 2019 to plot the mean ± standard deviation (SD) data.

Results

Allelopathic potential of decomposed solution of E. densa

The decomposed solution of *E. densa* has a specific allelopathic inhibitory effect on the germination of the highland barley seeds (Fig. 1). Among them, the decomposing solution for 30 d presents the greatest inductive effect. The GR and GI decrease as the decomposing concentration increases; both are lower than the control. Moreover, the decomposing solution has a delayed germination effect. With the prolongation of the decomposing time, the effect of the decomposing solution of *E. densa* on the germination of highland barley seeds weakens. Under the action of 25 mg/mL RD for 30 d, the GR of the

highland barley is the lowest. Except for 5 mg/mL, there are no significant differences in GR and GI between RD for 60 d of other treatments and the control ($P > 0.05$) (Fig. 1a, Fig. 1b). According to the SI, the decomposing solution with the shortest decomposing time has the strongest effect of decomposing liquefaction. The allelopathic effect of RD and the AD for 30 d is 2.56 times and 2.09 times the decomposed solution of the decomposed 60 d, and the liquefaction index of AD is 2.06 times the RD. The effect of the decomposing solution of *E. densa* on the rooting of highland barley has a time effect (Fig. 1). The decomposed solution for 30 d has the greatest effect on the rooting of highland barley, and the RR gradually decreases as the decomposed solution's concentration increases. Under the action of 25 mg/mL RD and 50 mg/mL AD for 30 d, the RR of the highland barley is 69.13% and 62.42% of the control group, respectively. There is no significant effect on the RR of the highland barley, while the AD presents an increasing-declining-increasing trend as the concentration increases. Furthermore, we find that as the decomposition time prolongs, the inhibitory effect of the decomposing solution on the rooting of the highland barley seeds weakens or even turns into a promoting effect. The allelopathic effects of the RD and AD for 30 d are 4.75 and 1.97 times the decomposing solution for 60 d, respectively, and the allelopathy intensity of the AD is 1.52 times that of the RD.

Figure 2 illustrates the effect of the decomposing solution of *E. densa* on the growth of highland barley seedlings. RD has no significant effect on the plant height and root length of the highland barley seedlings ($P > 0.05$). Under the AD action, the highland barley plant height increases in a time-dependent and concentration-dependent manner, while the root length shows a concentration-dependent decrease. In the 50 mg/mL treatment group, the plant height of the decomposing solution for 30 d and 60 d increased by 3.88% and 8.73%, respectively. However, the root length of the 50 mg/mL AD treatment is only 67.87% of that of the control (Fig. 2c). The effect of decomposing the solution on dry weight has a promoting effect, with a time-concentration dual effect. Among them, the highland barley seedlings treated with 50 mg/mL AD for 60 d are dried, with the weight being 1.3 times the control group (Fig. 2d).

The above study demonstrates that the negative effects of the decomposing process of the stumps of *E. densa* on highland barley are mainly manifested in the effects on seed germination and root growth.

Changes in the characteristics of RBCs of highland barley under the action of a decomposing solution of E. densa

After AO/EB staining, the RBCs in the control group showed green fluorescence, with a clear cell outline, evident nuclear structure, and green fluorescence bright spots. After treatment with the decomposing solution, the cells' nuclei are pyknotic, presenting some apoptosis (Fig. 3a), and the overall cell viability decreases (Fig. 3b). The shorter the decomposing time, the greater the cytotoxicity of the decomposing solution compared with the control, with the cell viability of the aerial parts decomposed for 30 d decreasing by 28.56%. At the same time, the decomposing solution treatment induced the thickening of the mucilage layer of the RBCs (Fig. 4a), but there was no significant difference from the control (Fig. 4b).

Effects of the decomposing process of E. densa **on soil properties and bacterial community**

The *E. densa* decomposition significantly increases the soil's pH ($P < 0.05$), with the 0.08 g/g treatment group presenting the largest pH increase after 20 d of decomposition. Over time, the growth amplitudes decrease, but all are higher than the pH of the control group (Fig. 5a).

In addition to having a minor effect on the SOM and TK content ($P > 0.05$) (Fig. 5b, Fig. 5e), other nutrients are changed to varying degrees during the decomposing process of *E. densa.* Compared with the control, the soil TP (Fig. 5c) and AP (Fig. 5d) contents increase, and the changes are the largest when decomposing for 20 d. The difference from the control decreases as the decay time increases but is still higher than the control. The effective potassium content of the treatment group is lower than the control and dose-dependent. The effective potassium content of the 0.08 g/g treatment for 40 d is only 1.92% of the control (Fig. 5f). The soil TN content increases significantly with the increase of the treatment dose ($P < 0.05$), and the maximum treatment dose (0.08 g/g) of decomposing for 20 d has the highest soil TN content, 1.20 times the control (Fig. 5g). The soil's AN content first decreases and then increases with the increase of the treatment dose, and decreases with the prolongation of the decomposing time (Fig. 5h).

The decomposing process of *E. densa* increases the activity of extracellular enzymes in soil. The urease activity shows an increasing-decreasing trend with the increase of the treatment dose and reaches a peak at 0.02 g/g (Fig. 6a). The decomposition time has a minor effect on the urease activity. The changes in sucrase, cellulase and polyphenol oxidase activities are the same, showing an increasing-decreasing trend with the increase of the treatment dose at 40 d and 60 d, and the activities of the three soil extracellular enzymes increases in a dose-dependent manner at 20 d of decomposing. The activities of sucrase (Fig. 6b) and polyphenol oxidase (Fig. 6e) increase the most in the 0.08 g/g treatment group at 20 d. In comparison, the cellulase increases the most at 0.02 g/g at 40 d (Fig. 6c). The acid phosphatase activity of the 60 d treatment group is significantly higher than the decomposing 20 d and 30 d treatment groups. However, there is no significant difference with the control (Fig. 6d), indicating that the changes in soil acid phosphatase activity are irrelevant to the decomposition of *E. densa*.

The α-diversity index of soil bacteria increases with the prolongation of the decomposing time of *E. densa* (Table 1). In different decomposing times, the PD value shows an increasing-decreasing trend with the increase of the residue content of *E. densa* in the soil. Except for the 0.08 g/g treatment decomposed for 20 d, the PD values of the other treatments are higher than the control. The number of observed species shows a decreasing-increasing trend with the prolongation of decomposing time. Among them, the 0.08 g/g treatment group at 20 d has the lowest observed species (1727) and reaches the highest (2485) at 60 d. The observed species in the samples are analyzed, except that the Shannon-Weiner index and Chao 1 index of the 0.08 g/g treatment in the 20 d decomposing treatment are lower than the control group. In contrast, the other treatments are higher than the control, indicating that the decomposing process of *E. densa* increases the level of bacterial community diversity. Except that the 20 d decomposing treatment group has a more significant difference within the group, the β-diversity of soil bacteria in the 40 d and 60 d is similar (Fig. 7).

Table 1
Bacterial α-diversity analysis in the decomposed soil of *E. densa*. The relevant indicators are Phylogenetic diversity, Chao 1 index, Observed species, and Shannon-Weiner index.

Time /d	Residue content g/g	Phylogenetic diversity	Chao 1 index	Observed species	Shannon-Weiner index
20	0	88.54 ± 4.26^{a}	3069.75 ± 132.91^{a}	2369.27 ± 120.39^{a}	9.31 ± 0.17^{a}
20	0.02	90.29 ± 3.6^{a}	3031.77 ± 219.18^{a}	2385.2 ± 123.18^{a}	9.44 ± 0.08^{a}
20	0.04	90.63 ± 3.27^{a}	3210.79 ± 48.66^{a}	2441.8 ± 62.02^{a}	9.51 ± 0.14^{a}
20	0.08	67.57 ± 0.55^{b}	2604.23 ± 22.75^{b}	1727 ± 31.42^{b}	7.87 ± 0.08^{b}
40	0	84.45 ± 1.7^{a}	2996.29 ± 122.86^{b}	2262.67 ± 7.74^{a}	9.05 ± 0.07^{a}
40	0.02	85.89 ± 3.87^{a}	3144.62 ± 81.79^{ab}	2293.6 ± 117.67^{a}	9.09 ± 0.27^{a}
40	0.04	88.36 ± 0.99^{a}	3309.81 ± 121.09^{a}	2404 ± 60.85^{a}	9.43 ± 0.11^{a}
40	0.08	86.47 ± 3.02^{a}	3164.17 ± 110.98^{ab}	2344.77 ± 75.83^{a}	9.38 ± 0.08^{a}
60	0	88.98 ± 0.78^{ab}	2965.78 ± 21.75^{b}	2395.77 ± 7.62^{b}	9.62 ± 0.07^{a}
60	0.02	91.76 ± 2.15^{a}	3212.95 ± 10.65^{a}	2479.3 ± 47.52^{a}	9.54 ± 0.16^{a}
60	0.04	92.53 ± 1.46^{a}	3230.53 ± 18.3^{a}	2485.95 ± 30.33^{a}	9.53 ± 0.11^{a}
60	0.08	86.34 ± 1^{b}	3189.56 ± 29.53^{a}	2349.4 ± 24.8^{b}	9.42 ± 0.04^{a}

A total of 30 bacterial phyla are detected in the decomposed soil of *E. densa*. During the decomposing process of *E. densa*, the six phyla Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes, Gemmatimonadetes, and Acidobacteria accounted for the highest proportion (Fig. 8). The relative abundances of Proteobacteria, Bacteroidetes, Acidobacteria and Gemmatimonadetes in the decomposed soil increase significantly with the prolongation of the decomposing time. In contrast, the relative abundances of Actinobacteria and Firmicutes decrease significantly. At 20 d and 40 d of decomposition,

the relative abundance of Proteobacteria shows a decreasing-increasing trend with the increase of residue content in the soil, while at 60 d of decomposition, it increases with the increase of residue content of *E. densa*. The trend is of high decrease, but still higher than the control. With the increase of the residue content of *E. densa*, the relative abundance of Bacteroidetes increased-decreased, the relative abundance of Firmicutes and Bacteroidetes decreased, and the relative abundance of Acidobacteria and Gemmatimonadetes increased with the prolongation of decomposing time.

The RDA redundancy analysis (Fig. 9) highlights that the soil chemical properties during the decomposing process of *E. densa* explain 51.56% of the bacterial community variability, and the two ranking axes explain 75.12% of the variability. Bacteroidetes and Proteobacteria are significantly positively correlated with urease, cellulase, sucrase, TN, AN, AP, and pH. Bacteroidetes and Proteobacteria were negatively correlated with acid phosphatase, AK, and TK. The Actinobacteria are significantly positively correlated with acid phosphatase, polyphenol oxidase, AK, TK, and SOM, and significantly negatively correlated with urease, cellulase, sucrase, TN, AN, AP, and pH. The Firmicutes have a significant positive correlation with AK and TK and a significant negative correlation with other environmental factors.

Discussion

Plants have a complex relationships with their neighbors, including competition, inhibition, stimulation, and interdependence (Zeng 2008). The Allelopathic active substances are contained in the organs of many higher plants and play an essential role in plant interactions (Sarheed et al. 2020). Allelopathic substances adversely affect plant seed germination, growth, and development by affecting some physiological processes in plants, such as cell division, photosynthesis, respiration, enzyme activity, and cell membrane permeability (Thiébaut et al. 2018, Mushtaq et al. 2019). Seed germination is the most sensitive growth stage for plants to external stress (Huang et al. 2020). Our study highlights that decomposing the stubble of the poisonous weed *E. densa* has an apparent inhibitory effect on the seed germination and young root growth of highland barley and exhibited concentration-dependent and delayed effects. The allelopathic inhibitory effect of the AD exceeds the RD. However, this negative effect is mainly reflected in the early decomposition stage, as in the late decomposition stage, the decomposing solution of *E. densa* has a significant promoting effect on the biomass accumulation of highland barley, manifested in the increase of dry weight, especially the dry weight of roots. The results reveal that the practical allelopathic components decrease continuously during the decomposition process of the stubble of *E. densa*, leading to the weakening of the later allelopathic inhibitory effect. Especially after the emergence of highland barley, the decomposed material of the stubble of *E. densa* provides the necessary nutrients for growing the highland barley seedlings and promotes the accumulation of dry matter of highland barley.

RBCs and their secretions form a protective structure, the root extracellular trap (RET), which is the first line of defense for plants to defend against microorganisms, heavy metals, and other harmful substances invading the root system (Ropitaux et al. 2020). When plants are under allelopathic stress,

their RBCs rapidly increase the thickness of the mucilage layer to resist harmful allelopathic substances (Ma et al. 2020). To a certain extent, changes in the thickness of the mucilage layer can represent the intensity of external stress. Our study shows that under the action of the decomposed substance of *E. densa*, the activity at the RBCs of the highland barley is reduced, the characteristics of apoptosis are observed, and the thickened mucilage layer is removed. These effects are similar to the decomposing solution effects on seed germination and root length. The shorter the decomposing time, the stronger the cytotoxicity of the decomposing solution. The cytotoxicity of the decomposing solution in the later stage of decomposing weakens or even disappears, and the cytotoxicity of the AD is greater than the RD. Combined with the experimental phenomenon that root growth is inhibited, it can be speculated that the decomposed substance of *E. densa* interfered with the defense function of RBCs, broke through the biological defense line, and affected the division and elongation activities of root tip cells to block root growth. However, the seedling growth is not affected significantly, and the substances produced by photosynthesis are transported to the roots, thereby increasing the root biomass.

E. densa is an annual aromatic herb, and the stems and leaves, especially the leaves, contain many volatile substances (Chauhan et al. 2018). Combined with the results of our study, we speculate that the effective allelochemicals of *E. densa* may be volatile terpenoids or aromatic compounds present in stems and leaves, especially in leaves. These substances are continuously dissipated during the decomposing process, and the main allelopathic substances in the decomposing substances gradually weaken or even disappear with the prolongation of the decomposing time. Therefore, the allelopathic effect of the decomposing process of the stump of *E. densa* is short, mainly occurring in the early stage. In the later stage of decomposition, with the reduction of the main allelopathic substances, the allelopathy of the decomposing substances weakens. The nutrients continuously decompose and are released, promoting the growth of the surrounding plants.

The interaction of plants-microorganisms-soil is the key to realizing plant community exchanges, changing biological diversity, and ecosystem functions (Mueller et al. 2020). As the core driving force of nutrient cycling and energy transformation (Shao et al. 2019), soil microbial communities are extremely sensitive to changes in the external environment, and their quantity and structural composition are affected by the soil environment and the dominant plant groups in the habitat (Yang et al. 2021). Decomposition of the stubble of *E. densa* led to an increase in soil pH, which effectively improves the living environment of soil bacteria (Li et al. 2022). Therefore, soil bacterial α-diversity increases significantly. At the same time, the bacterial community structure differs. The β-diversity analysis shows that the bacterial community changes the most in the early 20 d of decomposing, and there is little difference in other decomposing periods. This is consistent with the result that the allelopathic effect of *E. densa* on highland barley is mainly manifested in the early stage of decomposition.

Actinobacteria were always in a dominant position in the decomposing process of *E. densa.* It can accelerate the decomposition of animal (Araujo et al. 2020), and plant residues in the soil and increase the total nitrogen content in the soil with Bacteroidetes (Krishna et al. 2020). In addition, Bacteroidetes also have a phosphorus enrichment effect (Hou et al. 2021), which synergized with Actinobacteria

(Omotayo et al. 2021) with a phosphorus-dissolving effect to increase the content of AP in soil. Proteobacteria with increasing relative abundance during decomposition have nitrogen fixation and can adapt to various complex environments (Hou et al. 2019). In the late stage of decomposition, soil pH decreases, and the relative abundance of oligotrophic bacteria, Acidobacteria, which are suitable for growth in an acidic environment, increase and participate in the decomposition of organic matter and the balance of the micro-ecological environment (Wu et al. 2021). The relative abundance of Firmicutes continues to decrease, indicating that the carbon utilization rate continues to decline in the later decomposition stage (Uksa et al. 2017). This corresponds to an increase in the relative abundance of Acidobacteria. Acidobacteria and Gemmatimonadetes are more dominant in environments with lower moisture content (Gao et al. 2019). The increase in the relative abundance might have provided them with a more suitable habitat due to water consumption by microbial activities. Under the influence of such a complex and constantly changing bacterial influence network, there is no significant change in SOM.

Soil enzymes are mainly derived from soil microbial activities, plant root exudates, and enzymes released during the decomposition of animal and plant residues, which play an essential role in soil material cycling and energy transformation (Wu et al. 2020). In our study, hydrolase activities such as urease, sucrase, cellulase, acid phosphatase which are closely related to soil nitrogen, carbon, and phosphorus cycling (Wang et al. 2020) and redox-related polyphenol oxidase activities (Chen et al. 2020) are increased, indicating that the decomposition of the stubble of *E. densa* can release related enzymes to promote the synthesis of humus components in the soil and the flow and circulation of nutrient elements. We also observe no significant change in TK content but a significant decrease in the AK content. This may be related to the great demand for quick-acting potassium in the decomposition of *E. densa.* According to the resource allocation theory, soil microorganisms may also invest in abundant elements to produce extracellular enzymes to exploit relatively limited elements (Zhou et al. 2020).

Decomposition of *E. densa* stumps stimulates soil microbial activities by changing soil properties and extracellular enzyme activities. It jointly promotes the conversion of soil macromolecules into small molecules, enriching soil nutrients and ultimately leading to a significant increase in soil phosphorus and nitrogen content. Our hypothesis is further confirmed in the correlation redundancy analysis between soil bacterial diversity and chemical properties. *E. densa* has a huge underground root system. After enriching soil nutrient content, using a stronger root system to seize more soil resources (Lovera et al. 2021) may also be one of the means to accelerate its expansion.

Conclusion

Overall, during the expansion of the alpine meadow, *E. densa* releases allelochemicals through stubble decomposition and other pathways, interfering with the surrounding plants' defense function and directly inhibiting their growth. The stubble's decomposition changes soil microbial activities, enriches the soil nutrients, and forms a self-interested soil environment. Eventually, the *E. densa* gains an edge over the competition. The allelopathic mechanism of *E. densa* is illustrated in Fig. 10.

Declarations

Author contributions All authors conceived and designed research. XZ, YX, YX and HZ conducted experiments. DM, XZ, YX, YX, YW and YW contributed to the analysis and interpretation of data. ZX, XY and DM wrote and revised the manuscript. All authors read and approved the manuscript.

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Compliance with ethical standards

Competing interests The authors have no relevant fnancial or nonfnancial interests to disclose.

Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

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Figures

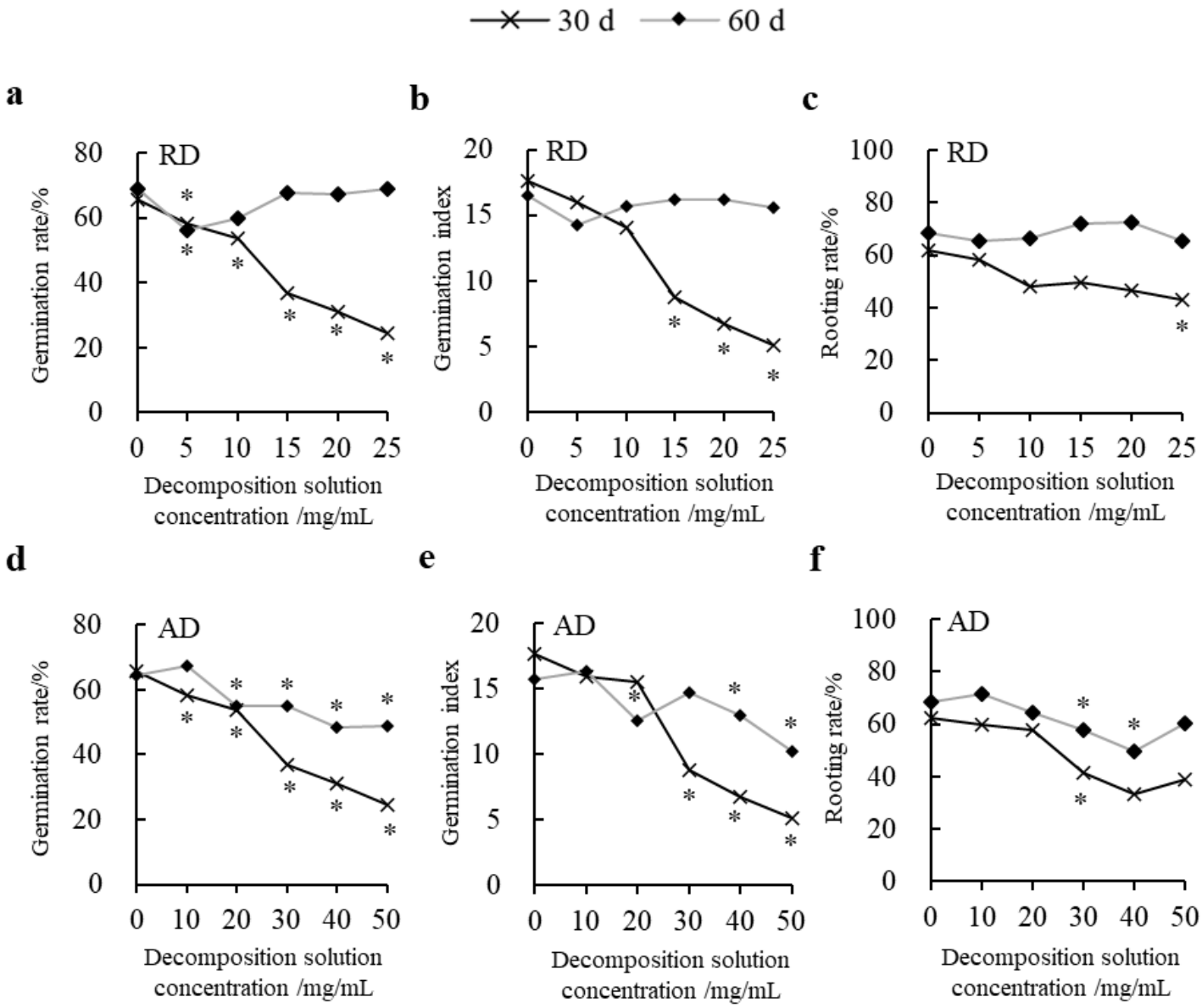

Figure 1

Variation trend of the highland barley seed germination and rooting under *E. densa* decomposing solution treatment. The decomposing part is marked on the upper right side of the Y-axis. "*" indicates the significant difference at the 0.05 level. "*" for 30 d and 60 d are marked below and above the broken line, respectively, the same below, (**a**)(**b**) *GR*, (**b**)(**e**) *GI*, (**c**)(**f**) *RR*

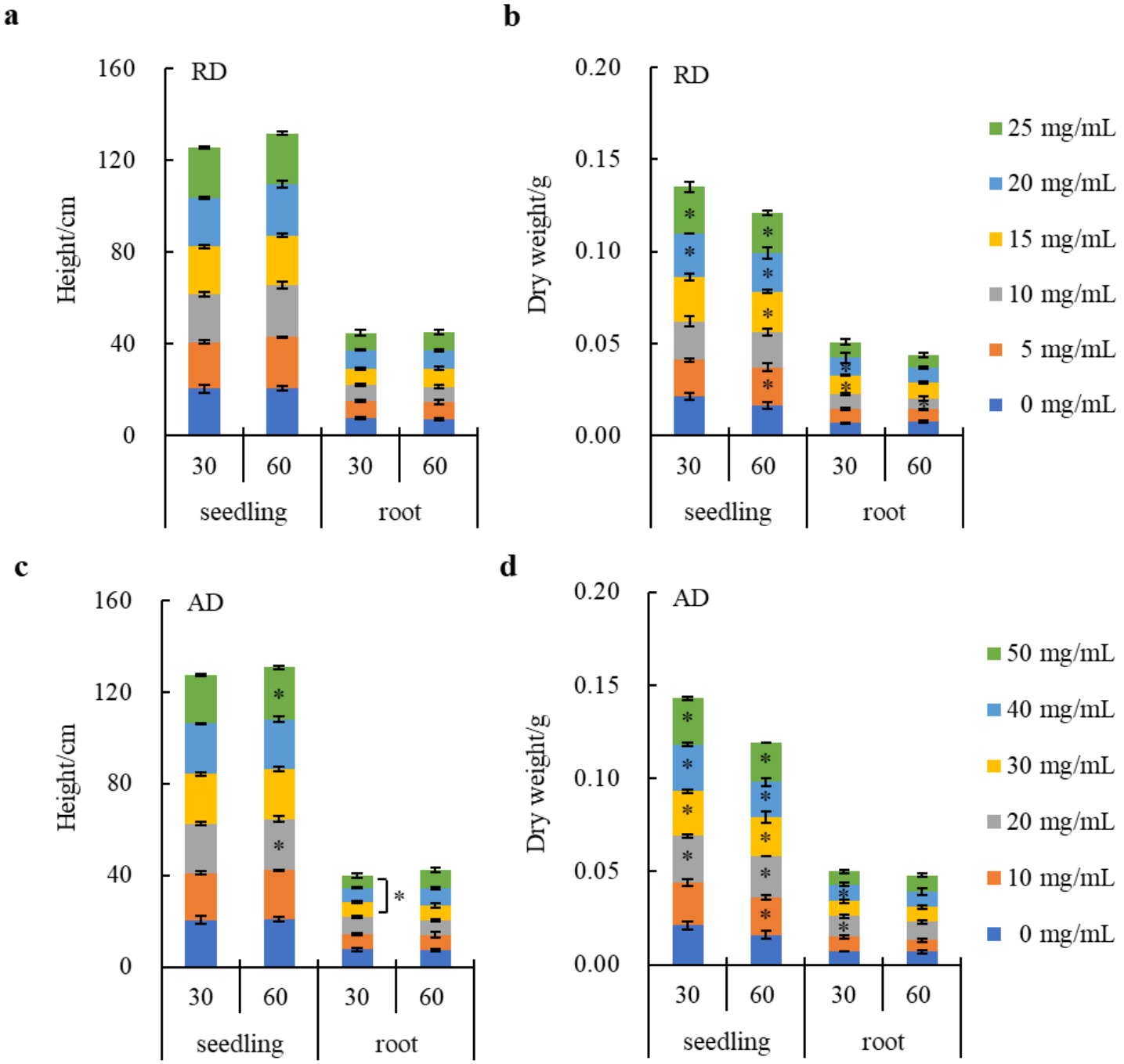

Figure 2

Changes in the growth indexes of highland barley seedlings under the action of *E. densa* decomposing solution. Data are represented as mean ± SD, the same below. (**a**)(**c**) height, (**b**)(**d**) dry weight

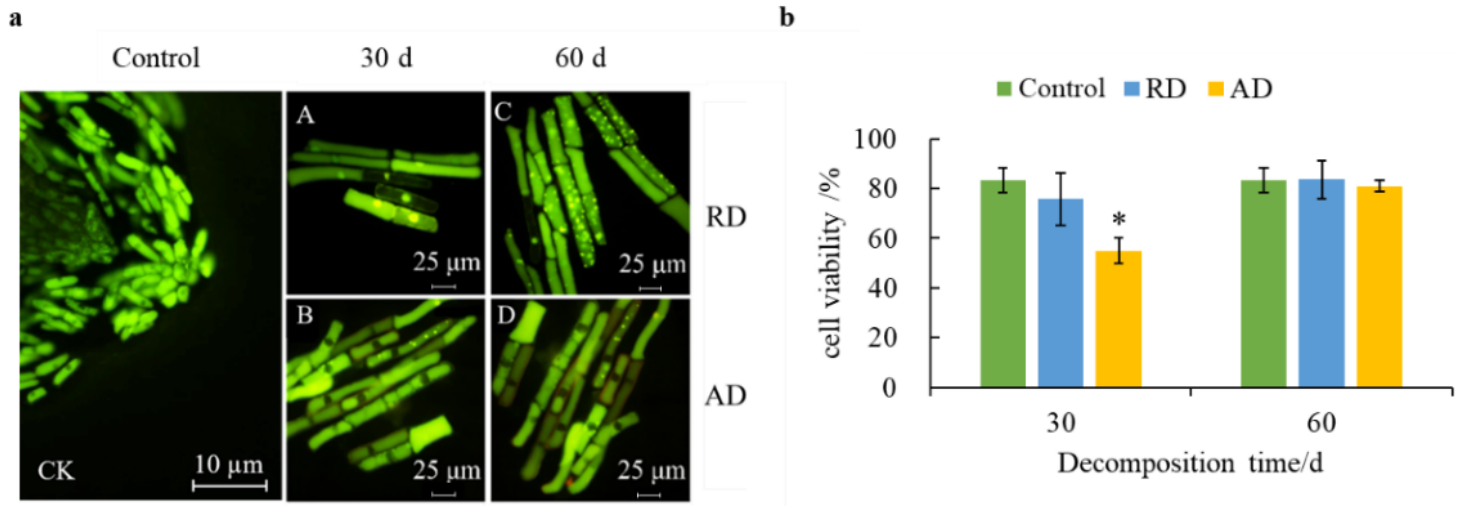

Figure 3

The effect of the decomposing solution of *E. densa* on the activity of the RBCs of the highland barley root. (**a**) the fluorescence micrograph under the action of the decomposing solution, green represents living cells, and orange-red represents cells that show the characteristics of cell death. (**b**) the activity of RBCs

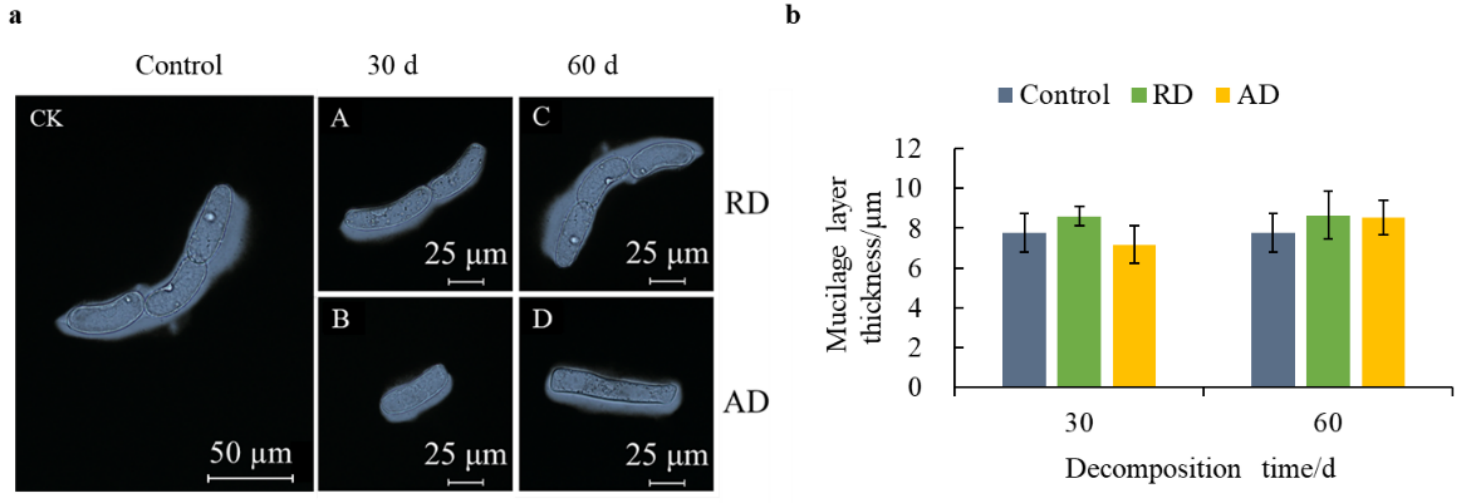

Figure 4

Changes in the thickness of the RBCs mucilage layer of highland barley under the action of the decomposing solution of *E. densa*. (**a**) the micrograph of the RBCs and their mucilage layer of highland barley under the action of the decomposing solution. (**b**) the variation trend of the thickness of the mucilage layer of RBCs

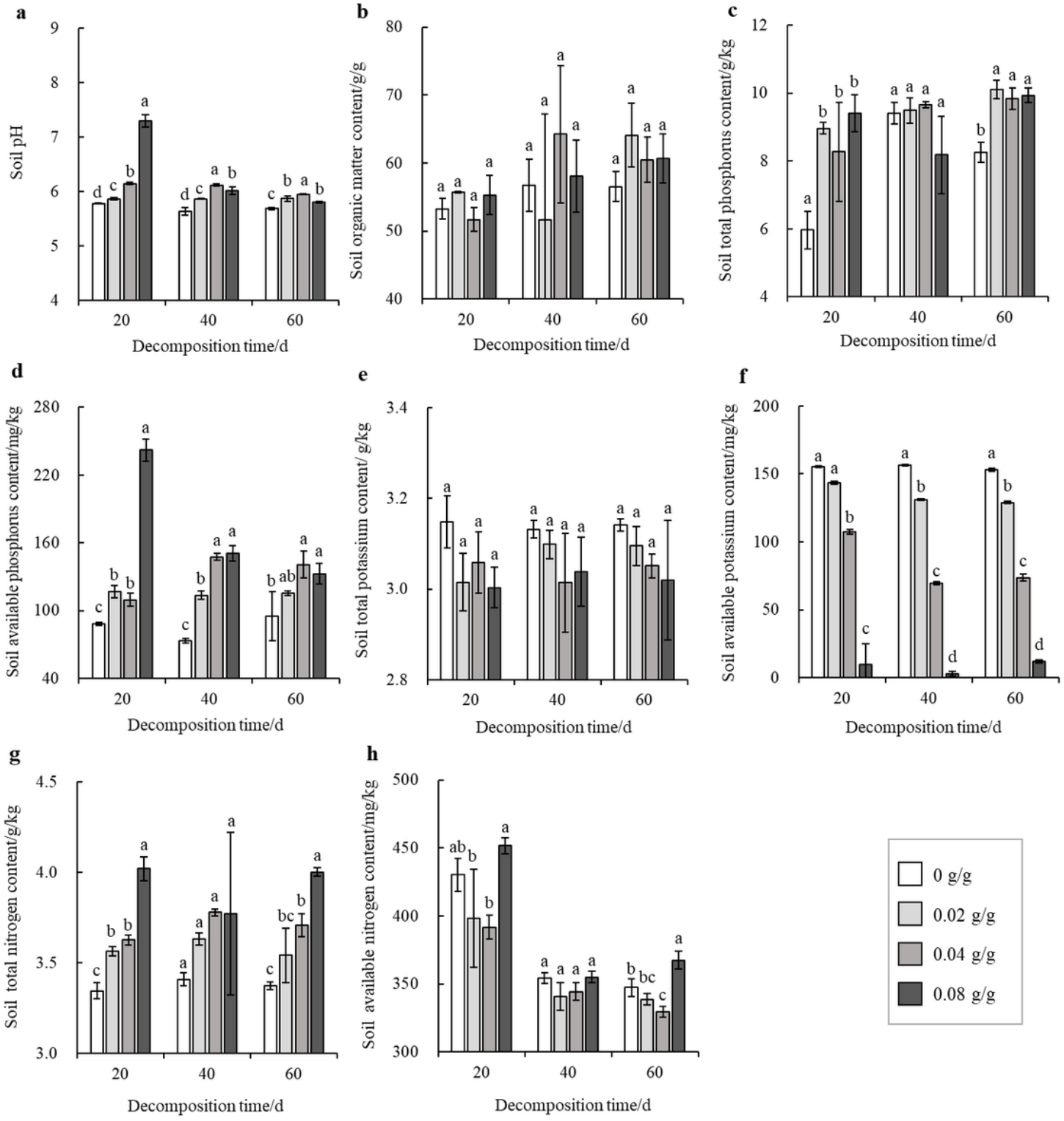

Figure 5

Effects of decomposing *E. densa* on soil pH and nutrients. The color in the column from light to dark indicates that the content of residues of *E. densa* increases, and different lowercase letters indicate the difference significant at the 95% level between different treatment gradients within the same treatment time, the same below. (**a**) soil pH, (**b**-**h**) the content of SOM, TP, AP, TK, AK, TN, and AN on the soil

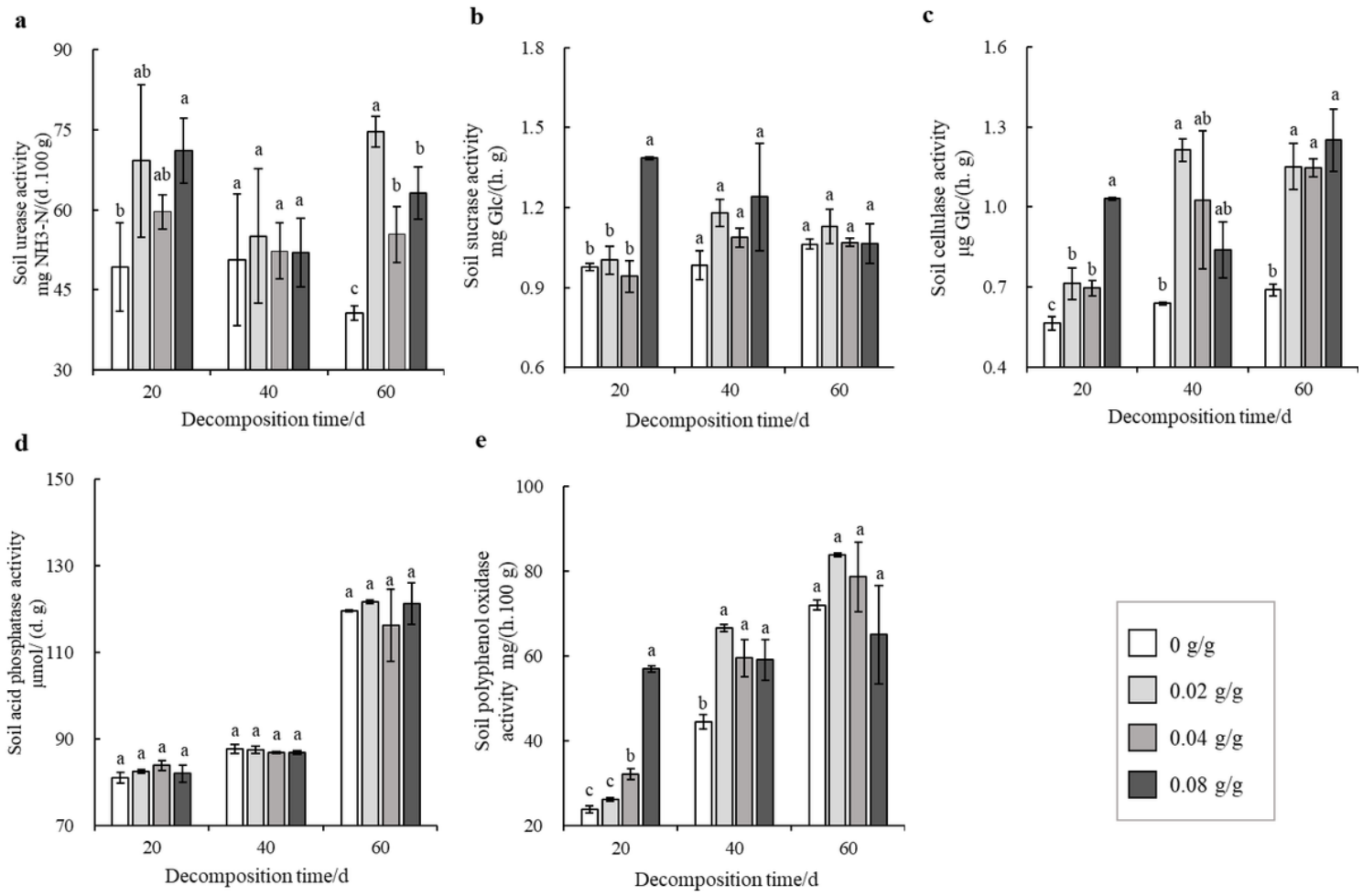

Figure 6

Effects of decomposed products of *E. densa* on soil enzyme activities. (**a**-**e**) the activities of soil urease, soil invertase, soil cellulase, soil acid phosphatase, and soil polyphenol oxidase

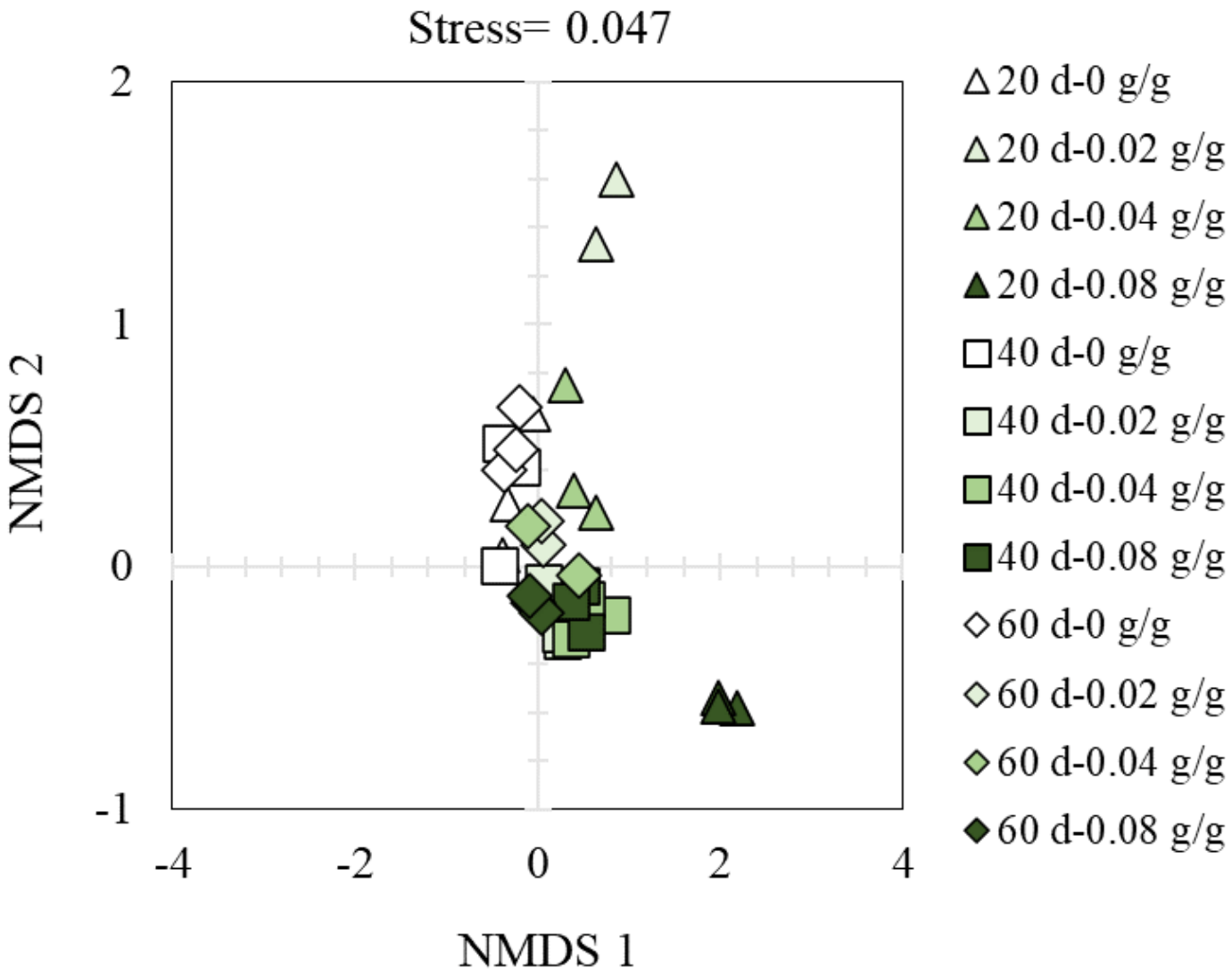

Figure 7

Bacterial β-diversity analysis of the decomposed soil of *E. densa*. The legend reads, "Decomposition d - the content of residues of *E. densa*", "△" indicates the 20 d treatment group, "□" is the 40 d treatment group, and " " is the 60 d treatment group. The green in the graph from light to dark indicates that the content of residues of *E. densa* increases.

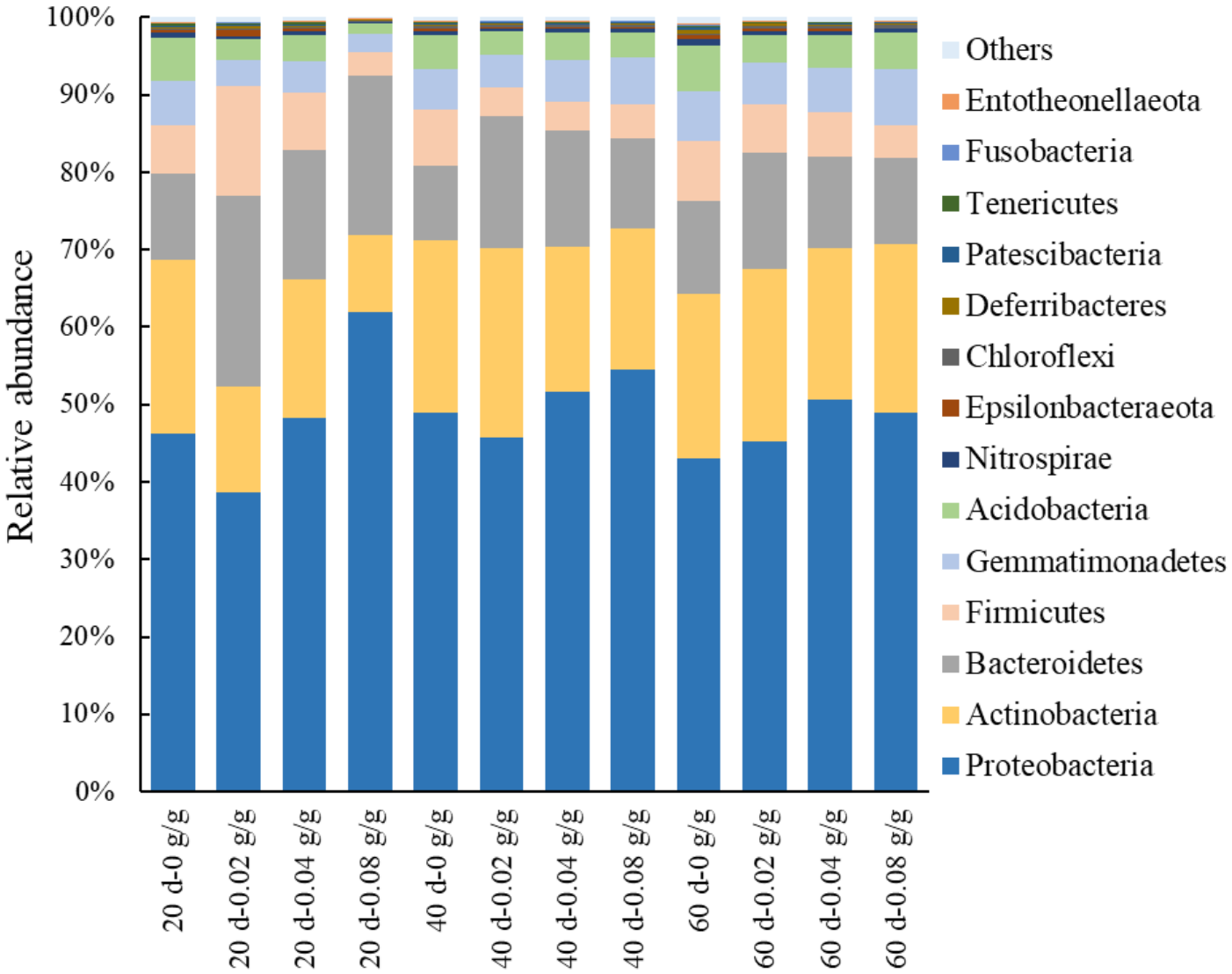

Figure 8

Relative abundance of bacteria at the phyla taxonomic level in the decomposed soil of *E. densa*

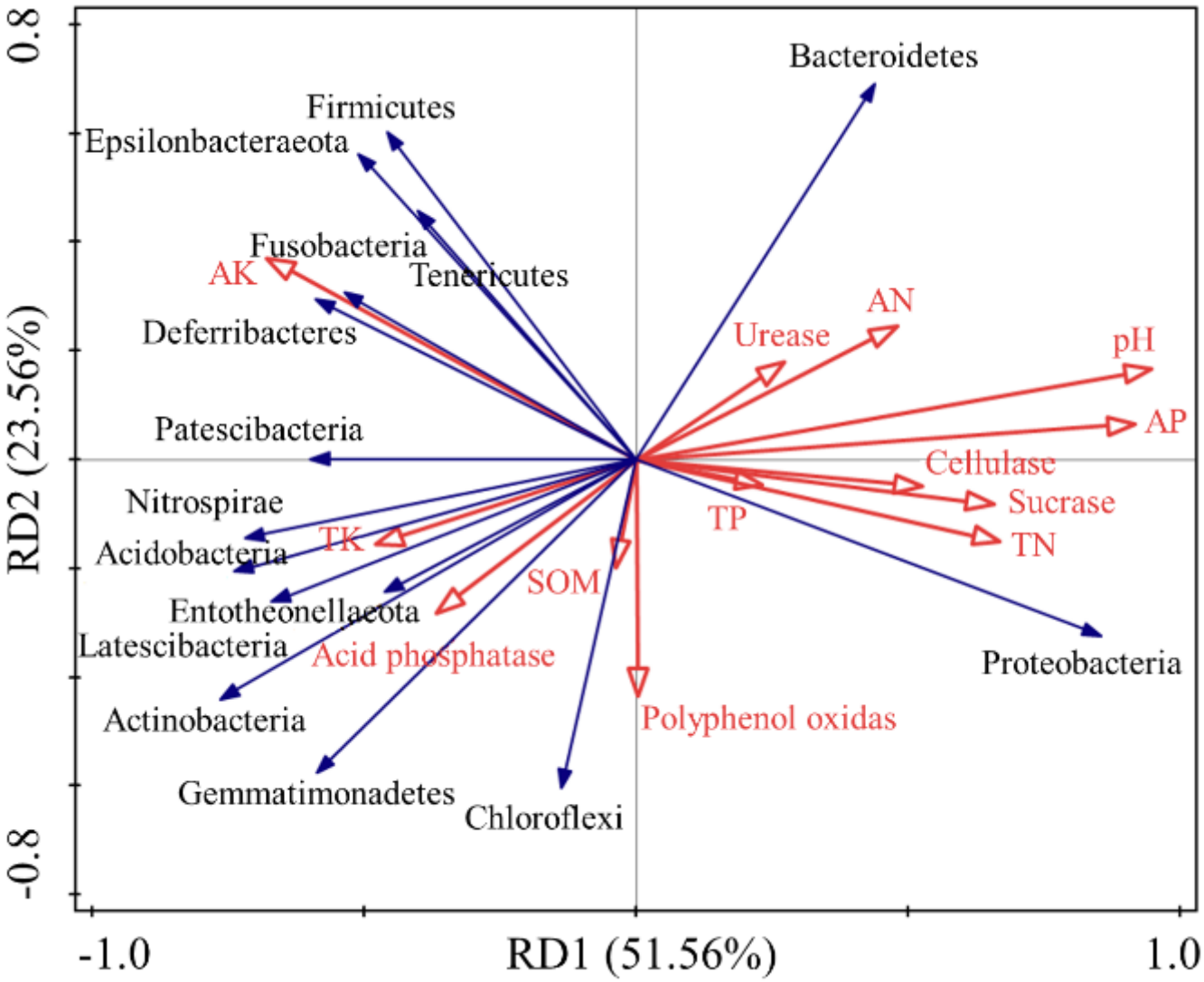

Figure 9

Correlation redundancy analysis between soil bacterial diversity and chemical properties during the decomposing process of *E. densa*. The red arrows represent different environmental factors, and the blue arrows represent different bacteria

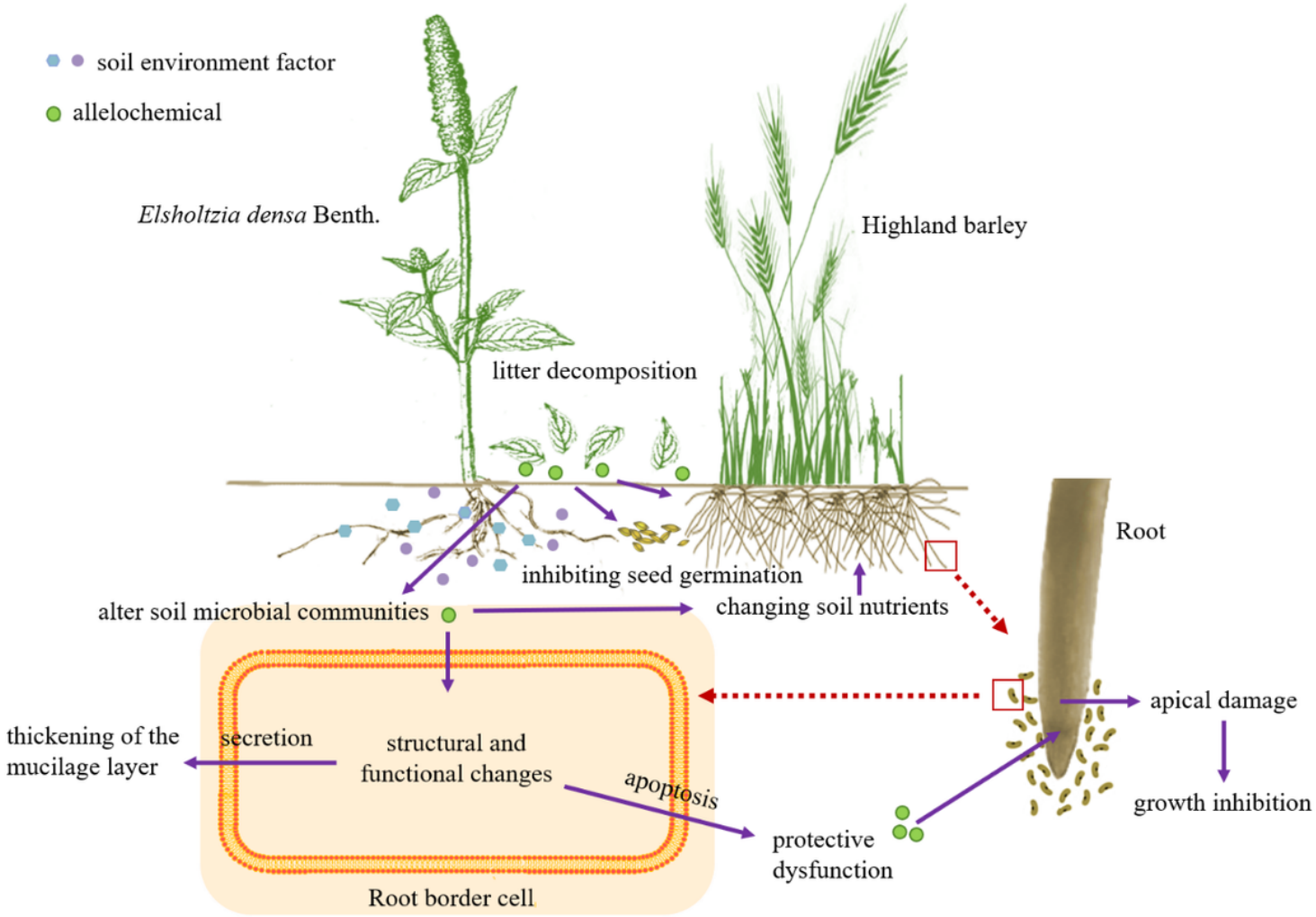

Figure 10

The expansion mechanism of *E. densa* through allelopathy