

Root ACC and rhizosphere ACC deaminase producing bacteria affects apple root architecture under soil compaction stress

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Research Article

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Abstract

Purpose To explore the relationship among root architecture, 1-aminocyclopropane-1-carboxylic acid (ACC) in roots and ethylene-modulating bacteria in rhizosphere and root endosphere under soil compaction stress, so as to provide theoretical reference for soil treatment.

Methods One-year-old potted apple (*Malus domestica* Borkh 'Red Fuji') trees as experiment materials, whose rootstocks were *Malus hupehensis* and *Malus robusta* respectively. After maintaining soil compaction stress for one year, we detected root architecture, root activity, root ethylene production, ACC content and the activity of ACC synthase (ACS) and ACC oxidase (ACO) in roots. Furthermore, we quantified the abundance of ethylene-modulating bacteria including the bacteria of ACC deaminase and ethylene-forming enzyme (efe) in rhizosphere and root endosphere, and analyzed their relationship with ethylene biosynthesis and root characteristics.

Results Soil compaction stress significantly changed root architecture, decreased root activity, enhanced ethylene production, increased ACC content and ACS activity in roots, regardless of whether the rootstock was *M. hupehensis* or *M. robusta*. The abundance of ethylene-modulating bacteria varied with rootstocks under compaction stress. Soil compaction stress decreased the abundance of ACC deaminase producing bacteria in *M. robusta* and increased that of efe in *M. hupehensis* both in rhizosphere and root endosphere. The changes of root characteristics were significantly correlated with ethylene production and ACC deaminase producing rhizobacteria.

Conclusion The increase of ACC content in roots and the decrease of abundance of ACC deaminase producing rhizobacteria jointly lead to the increase of ethylene release from roots, which lead to the change of root architecture under soil compaction stress.

Introduction

Plants produce ethylene under almost any stress. Ethylene is a gaseous hormone that has profound impacts on plant growth, development and stress response, moreover, it can also act as a bridge in the process of plant adaptation to environmental changes (Vandenbussche et al. 2012; Dubois et al. 2018). Low levels of ethylene enhance root and branch growth, while high levels of ethylene cause cell cycle arrest, inhibit cell expansion, promote plants dwarfing, and coordinate plant adaptation to the environment (An et al. 2010; Dubois et al. 2018).

ACC is an immediate precursor of ethylene, which can form ethylene under the action of ACC oxidase, and then regulate plant growth and development through the ethylene signaling pathway (Vanderstraeten and Van Der Straeten 2017). In addition, ACC independently acts as a signaling molecule to inhibit cell elongation, influence Arabidopsis growth and development (Tsang et al. 2011; Van de Poel and Van Der Straeten 2014; Tsuchisaka et al. 2009). ACS and ACO are two key rate-limiting enzymes in ethylene biosynthesis. These two enzymes respectively catalyze the synthesis and decomposition of ACC,

influence the response and adaptation of plants to stress by regulating the production of endogenous ethylene (Bleecker and Kende 2000; Alexander and Grierson 2002).

Microorganisms play an important role in the response and adaptation of plants to environmental stress. Some microorganisms can regulate plant physiology by secreting phytohormones, influencing endogenous phytohormones synthesis and signal transduction, so as to alleviate the effects of environmental stresses on plants (Zilber-Rosenberg and Rosenberg 2008; Ravanbakhsh et al. 2018). There are many microorganisms that can modulate ACC and ethylene levels in plant rhizosphere and root endosphere, such as ACC deaminase producing bacteria and efe producing bacteria. The two ethylene-modulating bacteria can respectively participate in ACC decomposition and ethylene production. They are the link between plants and soil, and can change the response of plants to environmental stress, the symbiosis between plants and microorganisms, even growth and development of the whole plant (Gamalero and Glick 2015; Nascimento et al. 2018; Bomle et al. 2021).

Increased soil compaction due to high mechanical loading, low species diversity, intensive grazing and improper irrigation, this is a major challenge for plant growth and development and modern agriculture (Shah et al. 2017; Bengough et al. 2011). Soil compaction limits the availability and transport of soil water and nutrients through increasing soil bulk density and reducing soil porosity, limits ethylene diffusion between root and rhizosphere, and inhibits root growth (Correa et al. 2019; Pandey et al. 2021). High compactness of soil enhances ethylene concentration. Ethylene induces auxin and ABA synthesis in rice roots, inhibit root elongation and promote root lateral expansion (Huang et al. 2022). Ethylene in soil can be derived from the biosynthesis of plant roots themselves, or the synthesis of rhizosphere and root microorganisms. In compacted soil, roots themselves cleverly "sense" soil mechanical resistance and "search" for loose sites, they can change morphology, slow elongation rate, regulate physiological metabolism, and recruit protective microorganisms against compaction stress (Zhang et al. 2021; Colombi et al. 2017). Among the microorganisms recruited by roots, some microorganisms are able to modulate ethylene levels through influencing ACC content and ethylene production (Glick 2005; Ravanbakhsh et al. 2018; North et al. 2020). These microorganisms may also contribute significantly to the increase of ACC and ethylene in roots under compaction stress.

Apple is widely cultivated fruit worldwide. Mechanical compaction, frequent trampling and improper soil management have resulted in increased soil compactness at many apple orchards. Soil compaction significantly inhibited root growth, reduced root activity and inhibited sulfur assimilation (Tian et al. 2020; Tian et al. 2021). *Malus hupehensis* Rehd. and *Malus robusta* Rehd. are common apple rootstocks. In this study, *M. hupehensis* and *M. robusta* as experimental materials, and increased soil compactness by repressing soil. After maintaining soil compaction stress for one year, we analyzed root characteristics, ethylene biosynthesis and the abundance of ethylene-modulating bacteria. Most importantly, to explore the relationship between root characteristics and ethylene biosynthesis and microorganisms under compaction stress, so as to provide theoretical reference for soil management.

Materials And Methods

Materials and experimental treatments

This experiment was conducted at the National Apple Engineering Technology Research Center of Shandong Agricultural University and the Key Laboratory of Fruit Tree Biology in University of Shandong Province from March 2021 to May 2022. The one-year old 'Red Fuji' apple saplings (*Malus domestica* Borkh 'Red Fuji'), whose rootstocks are *M. hupehensis* Rehd. (abbreviated as Mh) and *M. robusta* Rehd. (abbreviated as Mr) were planted in pottery pots (height 23 cm, diameter 32 cm) on 12 March 2021. Soil was prepared by mixing with cinnamon soil, organic fertilizer, and river sand at a 3:1:1 volume ratio. The soil organic matter content was $10.05\text{g}\cdot\text{kg}^{-1}$, total nitrogen content was $0.30\text{g}\cdot\text{kg}^{-1}$, total phosphorus content was $3.39\text{g}\cdot\text{kg}^{-1}$, total potassium content was $2.67\text{g}\cdot\text{kg}^{-1}$, alkali-hydrolyzable nitrogen content was $110.28\text{mg}\cdot\text{kg}^{-1}$, available phosphorus content was $44.69\text{mg}\cdot\text{kg}^{-1}$, available potassium content was $296.9\text{mg}\cdot\text{kg}^{-1}$, pH value was 6.5.

One month after planting, potted saplings with consistent growth were selected. The potting soil was compacted, so that the soil mechanical resistance was increased from $1.15 \pm 0.05\text{g}/\text{cm}^3$ (Normal soil, Ns) to $1.65 \pm 0.05\text{g}/\text{cm}^3$ (Compacted soil, Cs). Four treatments of potted apple saplings were formed, that is, Mh-Ns, Mh-Cs, Mr-Ns, Mr-Cs, each treatment was replicated four times and each experiment repeated 3 times ($n = 3$). Samples sampled on April 12, 2022, that is, samples were sampled at 1 year after treatment.

Rhizosphere soil and roots sampling

Root samples were prepared to determine ethylene biosynthesis index: roots were gently removed from soil and roots were rinsed in deionized water, then dabbed dry with absorbent paper. A part of roots was weighed and sealed in a gas cylinder for the determination of ethylene release capacity. The other part of roots was frozen in liquid nitrogen immediately, ground into powder, and stored in the refrigerator at -80°C for the determination of ACC content, ACS and ACO activity.

Rhizosphere soil and root samples were collected according to the method of Bulgarelli et al (2015) and Sun et al (2008). Removed about 2cm of topsoil, gently removed the roots along with the soil, shaken off loose soil, and divided into two portions. The first portion was used for bacterial analysis: fresh roots (length 6 cm, diameter 2 mm) were cut with sterile scissors and placed in a centrifuge tube of sterile PBS buffer, shaken and rinsed at 200rpm for 20min twice. The roots were removed, and the two washing solutions were combined. After centrifuging at 4000g for 20min, the precipitated soil was rhizosphere soil, which was stored at -80°C to determine bacterial abundance in the rhizosphere. The removed roots were cleaned and disinfected on its surface, and stored at -80°C for subsequent determination of the abundance of functional bacteria in the root endosphere. The second portion was used for the analysis of root activity and root architecture. To measure the parameters of root architecture, the root image was obtained by scanning roots after spreading, then root length, average root diameter, number of root tips and number of root branching were measured by Wanshen LA-S series image analyzer.

Determination of root activity and ethylene biosynthesis index

Root activity was determined by TTC (2, 3, 5-triphenyltetrazolium chloride) reduction method (Davidson et al. 2000). Ethylene release capacity (ET) was determined by reference to the method of Grodzinski et al. (1982). ACC content was determined by reference to the method of Lizada et al (1979). ACC synthetase activity was determined by reference to the method of Kato et al (2000). ACC oxidase activity was determined by reference to the method of Inaba et al (1995).

Determination of ethylene-modulating bacterial abundance in rhizosphere and root endosphere

Soil bacterial DNA was extracted using the E.Z.N.A.® Soil DNA Kit (OMEGA, USA). Root genomic DNA was extracted using the plant genomic DNA rapid extraction kit (Cat#DP3112, Wuxi Batek Biotechnology Co., LTD). The DNA concentration and purity were determined using a BioDrop, and the DNA integrity was detected by 1.2% agarose gel electrophoresis. DNA samples were stored at -20°C until use.

Plasmids were constructed by standard methods. (i) Obtained the target DNA fragment. The *acdS* gene specific primers of ACC deaminase producing bacteria were designed by Li et al. (2015): *acdS*-f3 (5'-ATCGGCGGCATCCAGWSNAAYCANAC-3') and *acdS*-r4 (5'-GGCACGCCGCCARRTGNCRTA-3'). The *efe* gene specific primers of *efe* producing bacteria were designed by Sato et al. (1997): ETH-F1 (5'-ATGACCAACCTACAGACT-3') and ETH-R3 (5'-TAAGCCACCAACATCGTC-3'). (ii) The target fragment was ligated to the pMD18-T vector (TaKaRa, Beijing, China) and transformed into *E. coli* DH5α competent cells. (iii) The positive clones were selected and sequenced to extract *E. coli* plasmids, which was used as the standard.

Serial 10-fold dilutions of standard plasmids were prepared for fluorescence quantification and to obtain standard curves. DNA samples were used as templates for q-PCR. q-PCR reaction system was 20 µl, including 10µL SYBR® Premix Ex Taq™ (2X), 0.4 µl upper and downstream primers, 1µL DNA template, 8.2µL ddH₂O. The reaction conditions were as follows: pre-denaturation at 95°C for 30s; denaturation at 95°C 5S; annealing extension at 60°C for 30s, 60 cycles. The gene copy number of functional bacteria in rhizosphere and root endosphere was calculated according to the curve.

Statistics analysis

Statistical analysis and Pearson's correlation analysis were performed with Microsoft Excel 2010 and IBM SPSS 20.0. Redundancy analysis was performed at Canoco 5. Statistical assessment was conducted by two-sided Student's *t* test for two groups ($P < 0.05$ was considered statistically significant). Microsoft Excel 2010, Origin 2021 and Canoco 5 software were used for graphing.

Results

Effects of soil compaction stress on root architecture and root activity of apple

Soil compaction stress decreased soil porosity (Fig. 1A-D). No matter whether the stock was Mh or Mr, compacted soil significantly changed architecture (Fig. 1E-L), increased average root diameter, reduced root length, root tip number, root bifurcation number, root weight (Fig. 1M-Q), forced root lateral growth, decreased root biomass and changed root configuration. In addition, the part of roots was grayish brown, and root activity decreased obviously under compaction stress, among them, when rootstock was Mh, the decrease was 69%, and when rootstock was Mr, the decrease was 20% (Fig. 1R).

Effects of soil compaction stress on root ethylene biosynthesis of apple

In Fig. 2, no matter whether the stock was Mh or Mr, ACS activity (Fig. 2A) and ACC content (Fig. 2B) of apple roots were significantly increased under soil compaction stress, while the ACO activity (Fig. 2C) was not significantly increased. Root ethylene production was considerably increased. The increase was higher when the rootstock was Mh (Fig. 2D).

Effects of soil compaction stress on ethylene-modulating bacterial abundance of apple rhizosphere and root endosphere

In rhizosphere and plant tissues, there are ACC deaminase producing bacteria that can decompose ACC, and efe producing bacteria that can promote ethylene biosynthesis. The two bacteria can directly or indirectly modulate ACC and ethylene levels. The abundance of *acdS* gene can be used to quantify ACC deaminase producing bacterial abundance, and the *efe* gene abundance can be used to quantify the abundance of efe producing bacteria (Sato et al. 1997; Li et al. 2015).

In Fig. 3, the abundance of *acdS* gene and *efe* gene varied with different rootstocks in rhizosphere and root endosphere under soil compaction stress. When rootstock was Mh, compaction stress significantly decreased the abundance of *acdS* gene in rhizosphere and root endosphere, but had no effect on *efe* gene (Fig. 3A, B, D, E). When the rootstock was Mr, compaction stress similarly decreased the abundance of *acdS* gene in rhizosphere, but increased the abundance of *efe* gene in rhizosphere and root endosphere, had no obvious effect on *acdS* gene in root endosphere (Fig. 3A, B, D, E).

Obviously, ACC deaminase producing bacteria and efe producing bacteria were different depending on the rootstock under soil compaction stress. Therefore, this study compared the abundance of *acdS* gene and *efe* gene, and analyzed the change of their ratio. It was interesting to find that the ratio of *acdS* gene to *efe* gene in rhizosphere and root endosphere of apple significantly decreased under compaction stress, no matter whether rootstock was Mh or Mr (Fig. 3C, F). This might be due to the abundance of ACC deaminase producing bacteria and efe producing bacteria in normal soil was in a dynamic balance, which was broken by compaction stress, so that the abundance of ACC deaminase producing bacteria was relatively reduced.

Relationship between ethylene biosynthesis in roots and ethylene-modulating bacteria in rhizosphere and root endosphere under soil compaction stress

In order to find out the relationship between ethylene biosynthesis and ethylene-modulating bacteria in apple rhizosphere and roots under soil compaction stress, we analyzed their correlation based on redundancy analysis and Pearson correlation. RDA analysis showed that the first two axis explained contribution rate of 75.71% and 13.03% respectively, the cumulative amount of information explained reached 88.74%. Therefore, the first two axis reflected the relationship between ethylene biosynthetic substances and ethylene-modulating bacteria, including the ACC content and ethylene production were main factors that influence ethylene-producing bacterial abundance ($P < 0.01$, Fig. 4A).

Results of correlation analysis showed that the relationship between ethylene biosynthetic substances and ethylene-modulating bacteria varied with rootstocks. In Mh roots, the increase of ethylene production was significantly correlated with the increase of ACS activity and ACC content, and the decrease of *acdS* gene abundance in rhizosphere and root endosphere (Fig. 4B). In Mr roots, the increase of ethylene production was not only correlated with the increase of ACS activity and ACC content, and the decrease of rhizospheric *acdS* gene abundance, but also strongly associated with an increase in *efe* gene abundance in rhizosphere and roots (Fig. 4C). Under soil compaction stress, the common characteristics of the two rootstocks were that the increase of root ethylene production was correlated with the increase of ACS activity, the increase of ACC content and the decrease of *acdS* gene abundance in rhizosphere. Root ACC content had the most significant contribution to ethylene production (Fig. 4B,C).

Relationship between apple root characteristics, ethylene biosynthesis and ethylene-modulating bacteria under soil compaction stress

In Table 1, root architecture, root fresh weight, and root activity of apple were not influenced by rootstock varieties, but were significantly influenced by compaction stress. Root length, root tip number, root bifurcation number, root fresh weight and root activity were negatively correlated with root ethylene production, and positively correlated with rhizospheric *acdS* gene abundance. Furthermore, average root diameter was positively correlated with root ethylene production, and negatively correlated with rhizospheric *acdS* gene abundance. These results indicated that the changes of root architecture, the decrease of root biomass and root activity under compaction stress were significantly related to ethylene production and the abundance of ACC deaminase producing bacteria in the rhizosphere.

Table 1

Correlation between root characteristics, ethylene biosynthesis and ethylene-modulating bacteria of apple under soil compaction stress

	Root architecture				Fresh root weight	Root activity
	Root length	Average root diameter	Number of root tips	Number of bifurcation		
Varieties	0.035	0.169	0.029	0.057	0.057	-0.111
Compactness	0.994**	-0.894**	0.954**	0.993**	0.963**	0.819**
ACS	-0.556	0.316	-0.526	-0.580*	-0.617*	-0.324
ACC	-0.606*	0.472	-0.554	-0.643*	-0.647*	-0.505
ACO	-0.383	0.326	-0.346	-0.375	-0.374	-0.195
ET	-0.875**	0.921**	-0.821**	-0.907**	-0.827**	-0.963**
Rh_acdS	0.735**	-0.857**	0.720**	0.719**	0.631*	0.859**
Rh_efe	-0.290	0.361	-0.280	-0.254	-0.237	-0.208
En_acdS	0.355	-0.315	0.297	0.406	0.417	0.439
En_efe	-0.521	0.452	-0.513	-0.472	-0.458	-0.250
** Significant correlation was found at the 0.01 level (bilateral); *Significant correlation was found at the 0.05 level (bilateral).						

Discussion

Soil compaction stress reduces soil porosity, increases root penetration resistance, and inhibits root growth (Correa et al. 2019; Zhang et al. 2021). This study finds that soil compaction stress significantly changes root architecture, reduces root activity, and similarly inhibits root growth of apple (Fig. 1). Root grown in compacted soil are similar to those exposed to ethylene (Sarquis et al. 1991), the reason was that soil compaction limited ethylene diffusion (Pandey et al. 2021), which lead to ethylene accumulation in root elongation zone cells, and then inhibited root growth through ABA and auxin (Huang et al. 2022). In this study, we found that root ethylene production increased significantly with the increase of soil compactness, and which is related to the changes of ethylene anabolism and the functional bacteria of regulating ethylene level in root microenvironment. Moreover, root characteristics changed by soil compaction stress were also correlated with ethylene anabolism and functional bacteria.

In environmental stimulation, ethylene is synthesized that ACS mediates synthesis of ethylene precursor ACC, and then completed by ACO transformation (Dubois et al. 2018). Compaction stress results in ACS activity increased and the accumulation of ACC content in roots (Fig. 2A, B). However, there is no significant change in ACO activity (Fig. 2C), this may be due to the low concentration of oxygen in

compacted soil. As a precursor of ethylene, ACC plays a crucial role in ethylene biosynthesis pathway. Correlation analysis further shows that ACS activity increased and the accumulation of ACC content are significantly correlated with root ethylene production increased (Fig. 4B, C). Therefore, under compaction stress, the increase of ACC content is the material basis for ethylene production in roots.

Microbiota is an important part of the immune system and metabolism of plants, which can directly relieve stress and balance physiological functions by regulating plant hormone levels (Bulgarelli et al. 2015). In rhizosphere and root microbial communities, there have functional bacteria that modulate ethylene biosynthesis levels (Ravanbakhsh et al. 2018). For example, ACC deaminase producing bacteria indirectly reduced ethylene levels by decomposing ACC, and efe producing bacteria directly increase ethylene levels by producing ethylene forming enzyme (Glick 2005; Belimov et al. 2009; Gamalero and Glick 2015). Moreover, in the process of plant growth and development and adaptation to abiotic stress, the two bacteria interact with ethylene biosynthetic substances (Nascimento et al. 2018). Therefore, under soil compaction stress, the two types of functional bacteria influence ACC content and ethylene production in apple roots, ACC content and ethylene production also influence the abundance of these two types of bacteria in apple rhizosphere and root endosphere (Fig. 4A).

Rhizosphere microbial community of apple varies with different rootstocks (Xu et al. 2020). In Mh, soil compaction stress significantly reduces the abundance of ACC deaminase producing bacteria in rhizosphere and root endosphere (Fig. 3A, D), while in Mr, compaction stress causes more efe producing bacteria to accumulate in the rhizosphere and root endosphere (Fig. 3B, E). Under compaction stress, the two functional bacteria of modulating ethylene levels also have common changes in these two rootstocks, such as the abundance of ACC deaminase producing rhizobacteria decreased in both rootstocks (Fig. 3A), and ACC deaminase producing rhizobacteria is significantly negative correlated with root ethylene production (Fig. 4B, C). These results indicate that apple roots may reduce the amount of ACC decomposition by reducing the abundance of ACC deaminase producing rhizobacteria, then generate more ACC by increasing the activity of ACS (Fig. 2), and finally lead to the accumulation of more ACC for ethylene biosynthesis. As a result, the amount of ethylene production from apple roots is greatly increased. In other words, during soil compaction stress, the increase of root ethylene production not only result from the increase of root ACC accumulation, but also from the decrease of ACC deaminase producing rhizobacteria, which enable more ACC to participate in ethylene biosynthesis, and the accumulation of ACC content contribute the most to the increase of ethylene release quantity (Fig. 4B, C). This inevitably leads to a significant increase in ethylene production from apple roots during soil compaction stress (Fig. 2D). In addition, the increase of ethylene production and the decrease of ACC deaminase producing rhizobacteria in significantly influence the change of root architecture, the decrease of root biomass and the decrease of root activity (Table. 1, summarized in Fig. 5).

Conclusion

In this study, soil compaction stress changes root architecture, decreases root biomass, reduces root activity and increases ethylene production of apple roots. q-PCR shows that the abundance of ACC

deaminase producing rhizobacteria is reduced in both stocks treated during compaction stress. Pearson correlation analysis shows that the increase of apple root ethylene production is caused by the accumulation of ACC content on the one hand, and the decrease of ACC deaminase producing rhizobacterial abundance causes more ACC to participate in ethylene biosynthesis pathway on the other hand, in addition, the accumulation of ACC content has the greatest effect on the increase of ethylene production. Ethylene production and the abundance of ACC deaminase producing rhizobacteria were important factors influencing root characteristics of apple.

Declarations

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Author contribution All authors contributed to the study conception and design. HY contributed to the study design; JL carried out material preparation, data measurements and analyses; BC and YC assisted in the operation of experiment. JL wrote the manuscript, and HY revised the manuscript. All authors read and approved the final manuscript.

Data availability Data are available on request from the authors.

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

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Figures

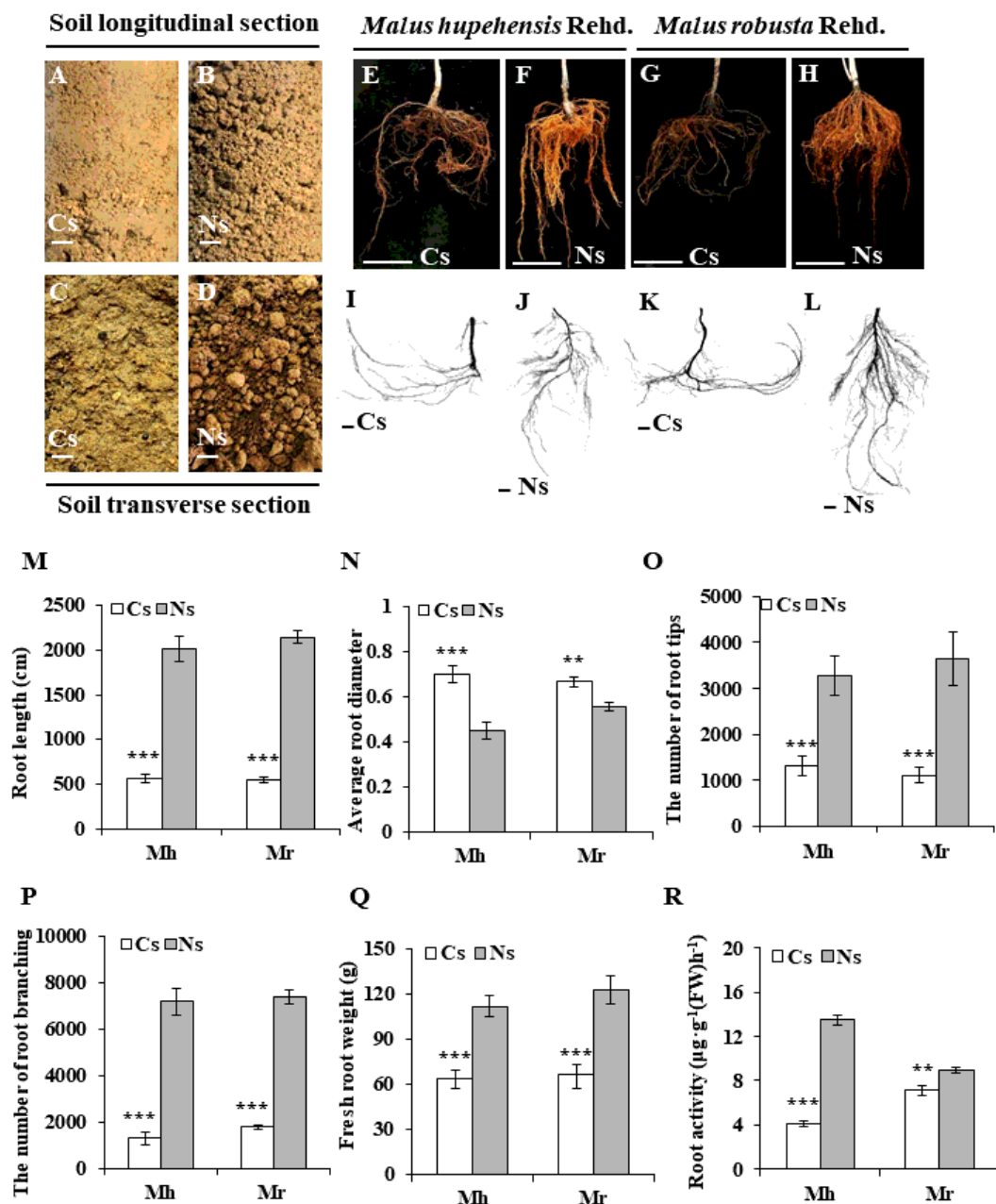


Figure 1

Soil compaction changes apple root architecture and reduces root activity. Cs refers to compact soil and Ns refers to normal soil. A and B are soil longitudinal sections, C and D are soil transverse sections, and the scale in the figure represents 1cm. (E-L) Complete root system (E-H) and partial root system (I-L) of the *M. hupehensis*Rehd. (Mh) and the *M. robusta* Rehd (Mr) in compact soil (Cs) and normal soil (Ns), the scale bar in E-H represent 10 cm, the scale bar in I-L represent 1 cm. (M-R) Root length (M), average

root diameter (N), number of root tips (O), number of bifurcation (P), fresh root weight (Q) and root activity (R) of *M. hupehensis* (Mh) and *M. robusta*(Mr) in compact soil (Cs) and normal soil (Ns). According to Student's t-test, all experiments were repeated three times, *, **, and *** represent *P* values of ≤ 0.05 , 0.01 and 0.001, the same below.

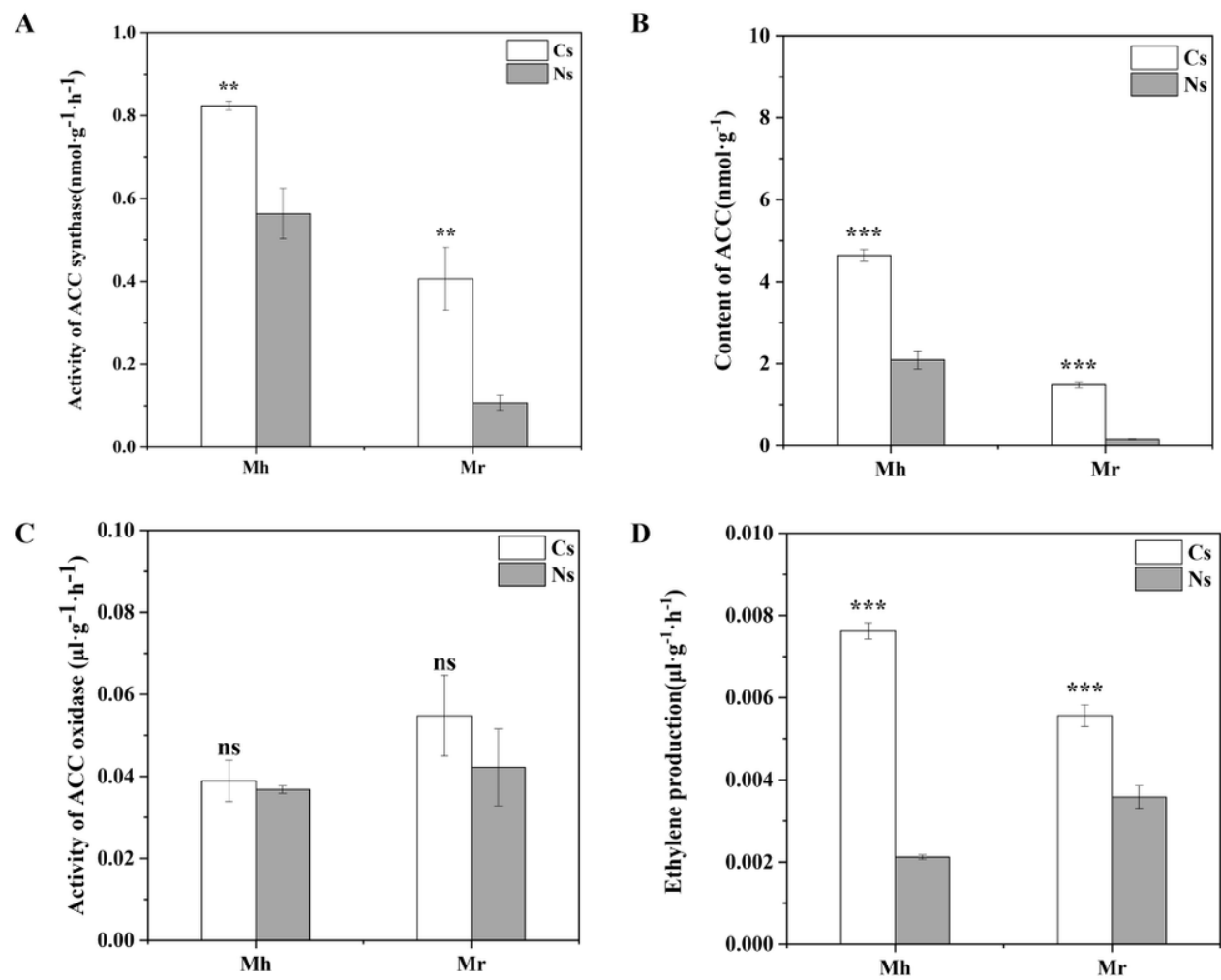


Figure 2

Root ACC synthase activity (A), ACC content (B), ACC oxidase activity (C), and ethylene release (D) of the *M. hupehensis* Rehd. (Mh) and the *M. robusta* Rehd (Mr) in compacted soil (Cs) and normal soil (Ns).

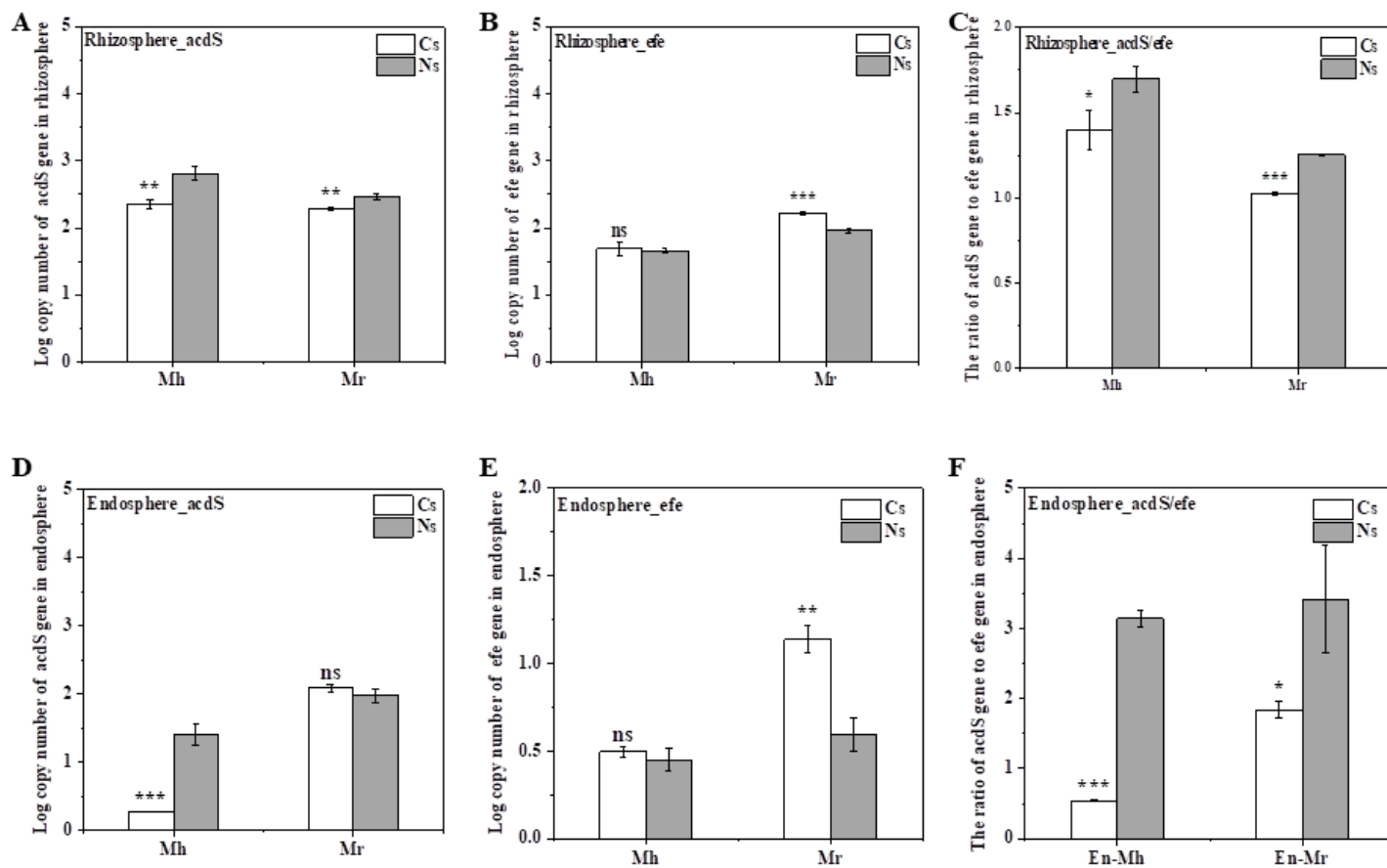


Figure 3

The copy number of *acdS* gene (A, D), the copy number of *efe* gene (B, E) and the ratio of *acdS* gene to *efe* gene (C, F) in rhizosphere and root endosphere of *M. hupehensis* (Mh) and *M. robusta* (Mr) under compacted soil (Cs) and normal soil (Ns).

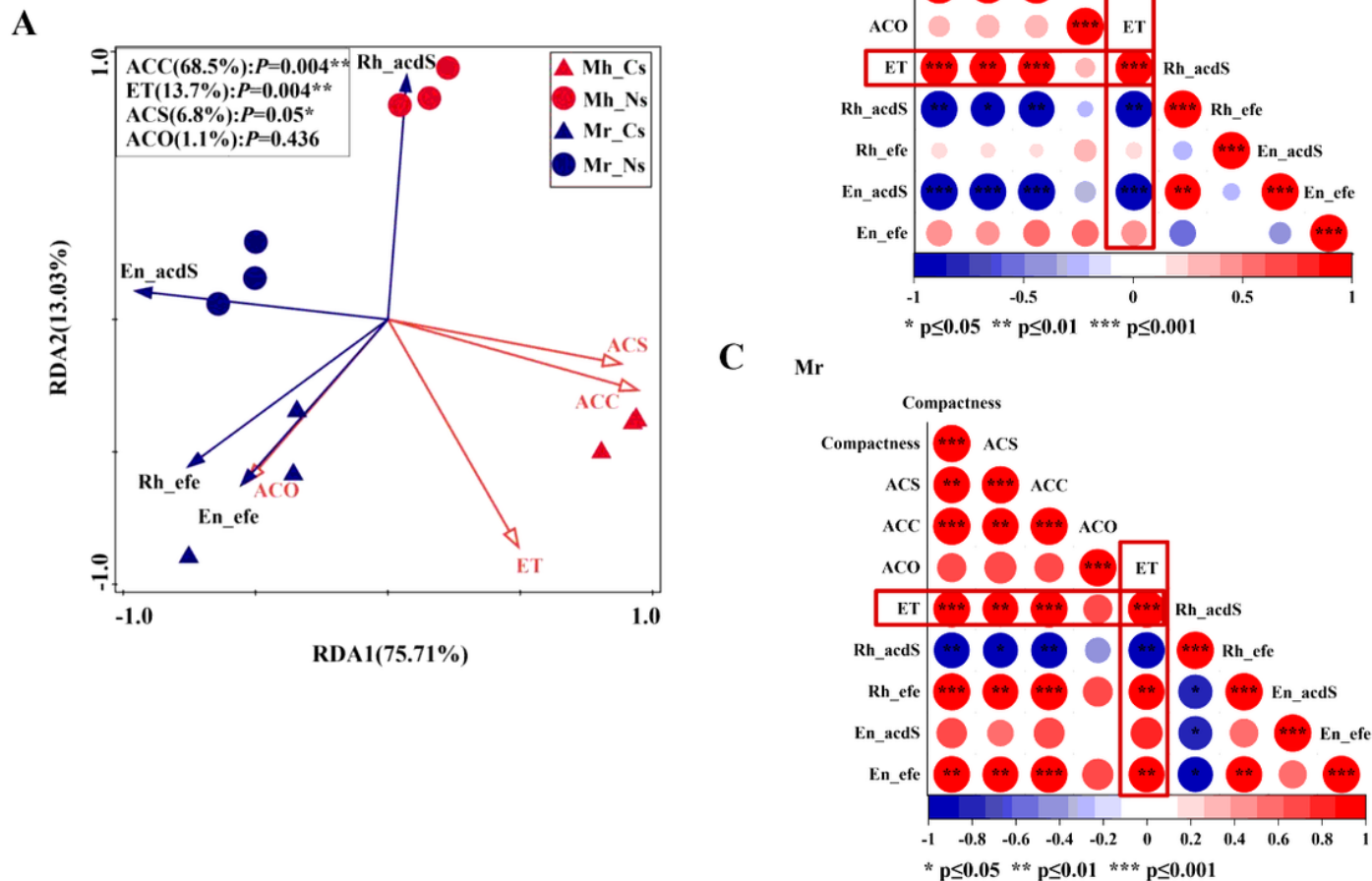


Figure 4

Redundancy analysis (A) and correlation analysis (B,C) between root ethylene biosynthesis and ethylene-modulating bacteria abundance. Redundancy analysis :the red arrows represent ethylene biosynthesis-related substances in roots, the blue arrows represent the bacterial abundance of ethylene biosynthesis functional genes in rhizosphere and root endosphere,symbols represent different sample distributions , the lengths of red arrows indicate the relative importance of ethylene synthesis-related substances in explaining functional gene abundance, and the angles between red and blue arrows indicate their correlations. Pearson correlation analysis: red represents positive correlation, blue represents negative correlation. Rh_acdS means acdS gene abundance in rhizosphere, Rh_eife means eife gene abundance in rhizosphere, En_acdS means acdS gene abundance in endosphere, En_eife means eife gene abundance in endosphere, the same below.

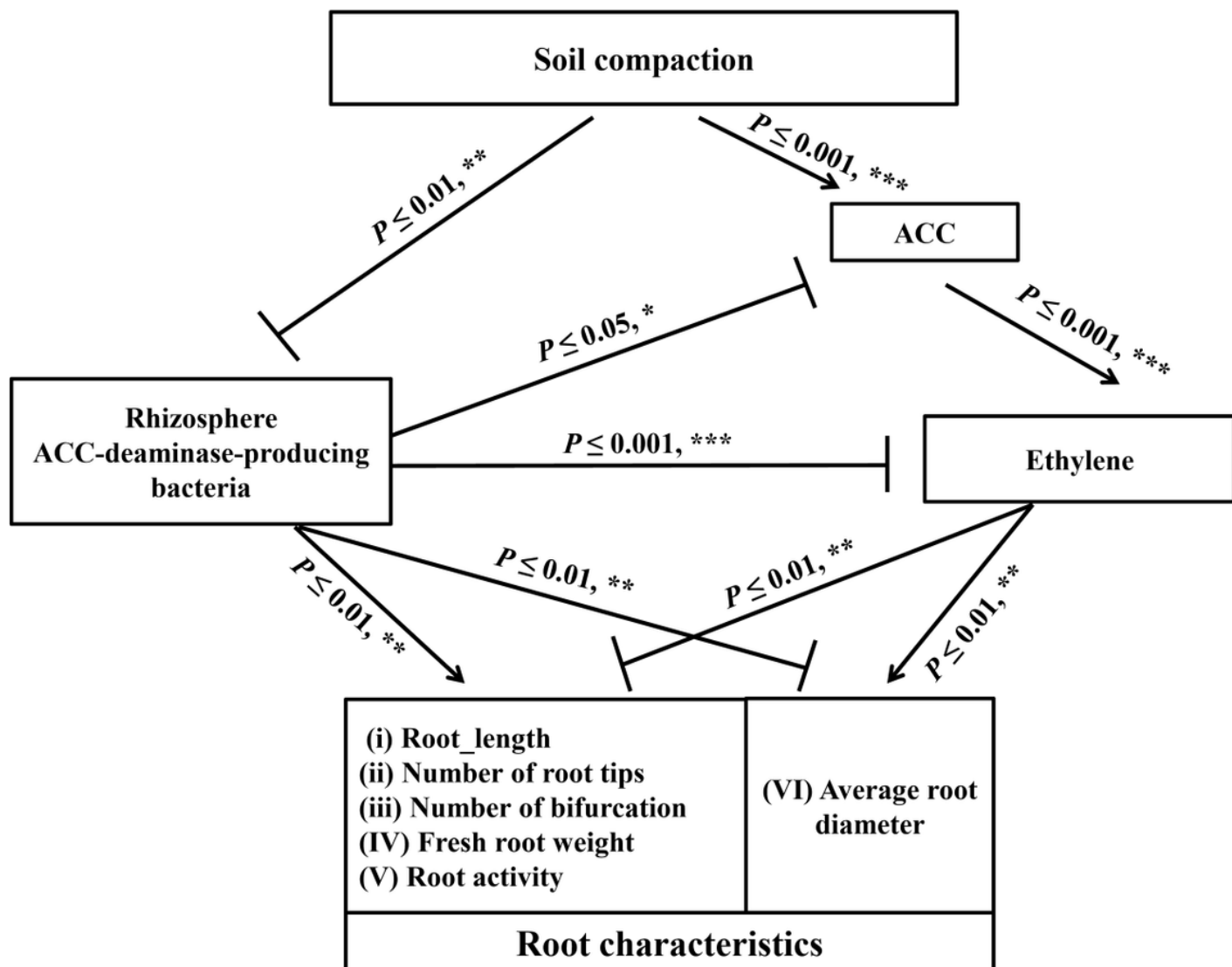


Figure 5

Under soil compaction stress, the summary diagram of the relationship among root characteristics, ethylene production, ACC content and rhizosphere ACC deaminase producing bacteria in apple