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# Plant roots sense soil compaction through restricted ethylene diffusion

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**Abstract:**

Soil compaction represents a major challenge for modern agriculture. Compaction is intuitively thought to reduce root growth by limiting their ability to penetrate harder soils. We report that root growth in compacted soil is instead actively suppressed by the volatile hormone ethylene. Mutant roots insensitive to ethylene penetrate compacted soil more effectively than wildtype. We demonstrate that roots sense mechanical impedance by employing the gaseous signal ethylene, as soil compaction lowers gas diffusion through a reduction in air-filled pores, causing ethylene to accumulate in root tissues and trigger hormone responses that restrict growth. We propose that ethylene acts as an early warning signal for roots to avoid compacted soils, revealing approaches to breed crops resilient to soil compaction.

**118/125 words**

**One Sentence Summary:**

Roots sense soil compaction employing the gaseous signal ethylene.

Soil compaction impacts global crop cultivation by reducing root penetration in both the upper and deeper soil layers (*1*). Modern agricultural practices have exacerbated soil compaction, largely due to intensification of operations leading to the deployment of heavier machinery and tillage practices (*2, 3*), severely degrading ~ 65 million hectares of land globally (*4*). Compaction increases soil bulk density and reduces soil porosity, limiting the availability and transport of water and nutrients (*4, 5*). The decrease in soil pore space, especially in large air-filled pores (Fig. 1, A-D; figs. S1 and S2 and *Movie S1 and S2*) also restricts diffusion of gases between roots and the rhizosphere (*6*). To deal with compacted soils and penetrate cracks, roots are reported to undergo adaptive growth responses, including increasing radial expansion of root tips (*1*). However, the predominant response of roots is cessation of growth of which the mechanistic basis remains unclear. Here, we report that entrapped ethylene functions as a key signal regulating root growth in compacted soils.

Ethylene is produced by root tissues and its level increases when roots are exposed to compacted soil (*7, 8*). Ethylene concentrations outside the root could increase due to the reduction in soil pore space in compacted soil, impacting gas diffusion from root tissues (Fig 1A-D; figs. S1 and S2). To test this 'restricted gas diffusion' model, we used the EIN3-GFP Arabidopsis ethylene response reporter (*9*; fig. S3, A-C) and examined the effect of covering root tips with a gas impermeable barrier. In agreement with model assumptions, restricting gas diffusion from root tip tissues triggered a rapid and sustained increase in EIN3-GFP in root elongation zone cell nuclei compared to controls (Fig. 1, F versus E; fig. S3, D-G). This result is consistent with (a) limitation of ethylene release from root tip tissues and (b) changes in gas diffusion rate between roots and the external environment inducing ethylene accumulation and signalling. To rule out that changes in ethylene signalling were related to reduced oxygen levels in root tip tissues, we treated roots expressing hypoxia markers *pPCO1:GFP-GUS*, *pPCO2:GFP-GUS* (*10*) and *RAP2.12-GFP* (*11*) with the gas impermeable barrier. Hypoxia reporters were not induced by the gas barrier but were induced by submergence (figs. S4 to S6). We conclude that EIN3-GFP induction results from restricted ethylene diffusion, rather than hypoxic conditions (*11*).

Roots exposed to elevated levels of ethylene exhibit growth inhibition (Fig. 1, I and J) which phenocopies the impact of soil compaction (Fig. 1, G and H). We observed that rice roots grown in 1.1 g $cm^{-3}$ (uncompacted) versus 1.6 g $cm^{-3}$ (compacted) soil bulk densities exhibit reduced root length when exposed to compacted conditions (fig. S7, A and B). Root anatomical analysis revealed that compaction caused a three-fold decrease in epidermal cell length (fig. S7C), matched by a three-fold increase in cortical cell diameter (compare Fig. 1, G and H, and fig. S7D). Similarly, ethylene treatment reduces root length (fig. S8A) whilst increasing root width (Fig. 1, I and J), by decreasing epidermal cell length and increasing cortical cell diameter (fig. S8, B and C).

To directly test the functional importance of ethylene during soil compaction, we examined root growth responses of wildtype (WT) rice versus ethylene insensitive mutants *osein2* and *oseil1* (*12*). *OsEIN2* (*ETHYLENE INSENSITIVE2*) encodes a key ethylene signaling component (*13*). *OsEIL1* (*EIN3-like 1*) encodes a critical transcription factor in the ethylene transduction pathway downstream of OsEIN2 (*9*). Mutations in rice *OsEIN2* and *OsEIL1* genes confer ethylene insensitive root elongation phenotypes (*12*; fig. S9, A and B). To analyse the impact of soil compaction on WT rice versus *osein2* root growth, lines were grown in columns either entirely filled with uncompacted soil (1.1 g $cm^{-3}$) or highly compacted soil (1.6 g $cm^{-3}$ with a 1cm top layer packed 1.1 g $cm^{-3}$ to help establish seedling root growth). Penetrometer resistance analysis demonstrated that root elongation rate is sensitive to increased soil strength (fig. S10).

To quantify the impact of soil compaction on root length of WT versus ethylene mutant lines, we employed the non-invasive X-ray imaging approach, Computed Tomography (CT; Fig. 2, A to G). CT imaging revealed that, unlike WT (Fig. 2B), both *osein2* and *oseil1* roots were able to penetrate highly compacted soil (Fig. 2, D and F; quantified in Fig. 2G). This result reveals ethylene signalling is critical for triggering root growth responses upon soil compaction. Anatomical analysis of rice mutant roots further demonstrated that under compacted soil conditions, *osein2* and *oseil1* root epidermal cells continued to elongate normally, whilst cortical cells did not undergo radial expansion (figs. S11 and S12) compared to WT (fig. S13). Moreover, this growth response also occurs in other classes of roots, since primary and lateral root growth and cortical responses induced by soil compaction are blocked in the ethylene insensitive *Arabidopsis* mutant *etr1* (figs. S14 to S17). Similarly, ethylene insensitive mutants in rice (*osein2* and *oseil1*) and *Arabidopsis* (*ein3eil1*) accumulated significantly higher shoot and root biomass in compacted soil conditions compared to WT (figs. S18 and S19). Hence, our rice and *Arabidopsis* mutant analysis reveals ethylene plays an inhibitory role in both monocot and eudicot root (and shoot) tissues when experiencing soil compaction.

Our results suggest that reduced root growth triggered by soil compaction does not arise from mechanical impedance, but instead represents a timely response controlled by ethylene, perhaps to avoid growth in compacted soils (*14*). To discriminate between the effects mediated by mechanical impedance versus ethylene, we compared their impact on root tip shape. Soil compaction causes WT rice roots to double in width and their root cap to develop a ‘flattened’ shape (compare Fig. 2, H and I). Soil compaction-induced radial growth and root cap shape changes were blocked in *osein2* (Fig. 2, J and K, and O). Hence, root tip shape changes induced by soil compaction appear to be controlled primarily by ethylene and not by mechanical impedance. Indeed, ethylene treatment alone was sufficient to trigger equivalent changes in root width (Fig. 1, I and J, and fig. S8, B and C) and cap shape (Fig. 2, L to N, and fig. S20 similar to roots exposed to soil compaction. Therefore, ethylene represents a critical signal in plants controlling shape changes underpinning root compaction responses.

Given ethylene's functional importance during root responses to compaction, we investigated whether soil mechanical impedance triggered increased ethylene signaling in root tissues. We employed transgenic Arabidopsis and rice either expressing an ethylene biosensor featuring *EIN3* (*9*) or *OsEIL1* sequences fused with GFP (fig. S21). In uncompacted soil, *35S:EIN3-GFP* or *proOsEIL1:OsEIL1-GFP* reporters in root nuclei were not detectable (Fig. 3, A and D). However, when reporter lines are grown in compacted soil, both ethylene reporters were detected in root elongation zone cells (Fig. 3, B and C, and E). To probe the role of ethylene in other soil types, we grew rice reporter lines in two other soils. Compaction triggered a root ethylene response in clay soil (figs. S21 and S22), and sandy loam soil (Fig. 3E, and fig. S23). Hence, the ethylene-based compaction mechanism appears to operate in different soil types.

How does soil compaction induce elevated ethylene signaling in root tissues? Mechanical impedance could cause roots to upregulate ethylene synthesis. Profiling of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) in excised rice root tips detected no change in levels after growth in compacted soil versus non-compacted controls (fig. S24). Alternatively, plant roots may sense soil compaction by monitoring ethylene levels. Mathematical modelling predicted slower outward ethylene diffusion rates under compacted soil conditions (Fig. 3H and fig. S25) due to the decreased volume of air-filled pores (*1*; Fig. 1, A-D and *movie S1* and *S2*). This will result in a higher ethylene concentration close to roots (Fig. 3, F and G) and therefore in root cells, consistent with soil compaction triggering an ethylene response (Fig. 3, B, C and E).

We directly tested whether soil compaction restricted gas diffusion by experimentally measuring ethylene's ability to move through compacted versus uncompacted soil. A 1cm thick soil column (connecting two air-filled chambers) was either left empty (control) or filled with uncompacted soil (1.1 g $cm^{-3}$) or compacted soil (1.6 g $cm^{-3}$) (Fig. 3I and S25B). Ethylene was injected into the upper chamber (an increase in pressure was avoided) and ethylene concentrations were subsequently measured over time in the lower chamber until an equilibrium was reached between the chambers. In agreement with gas diffusion simulations, ethylene levels rapidly reached an equilibrium with the lower chamber in control conditions without soil resistance (Fig. 3I). Ethylene was also able to diffuse through uncompacted soil, albeit 10-50 times more slowly than the empty control (Fig. 3I). In contrast, ethylene was unable to diffuse through compacted soil, and was still undetectable in the lower chamber at 20 days (Fig. 3I). This result demonstrates that soil compaction and the associated increase in soil moisture, due to less porosity, impacts ethylene diffusion rates, consistent with our 'restricted gas diffusion' model. This much slower ethylene diffusion in compacted soil results in an enhanced ethylene response in root cells. This entrapped ethylene gas provides a fast and reliable signal for plants to interact with their environment since nearly all roots produce ethylene under normoxic conditions (*15*).

Our results reveal how roots regulate growth responses to soil compaction. First, the inhibition of root growth by compacted soils is triggered by ethylene signalling, rather than simply by mechanical forces. Second, rather than using a dedicated mechano-perception mechanism, roots appear to sense soil compaction through restricted diffusion of this gaseous signal from the plant cells to the soil, causing ethylene to accumulate in root expansion zone cells, and inhibiting elongation growth. Third, compaction and soil moisture status appear to impact root elongation, not only because they control soil strength, but also through regulating ethylene diffusion. Fourth, we propose that ethylene acts as an early warning signal for roots to avoid compacted soils *(14)* providing a pathway for how breeders could select crops resilient to soil compaction.

**REFERENCES AND NOTES**


1. J. Correa, J. A. Postma, M. Watt, T. Wojciechowski, *J. Exp. Bot*. ***70***, 6019-6034 (2019).
2. B. D. Soane, P. S. Blackwell, J. W. Dickson, D. J. Painter, *Soil Till. Res.* ***2***, 3-36 (1982).
3. S. Mangalassery, S. Sjögersten, D. L. Sparkes, C. J. Sturrock, J. Craigon, S. J. Mooney, *Sci. Rep.* ***4***, 1-8 (2014).
4. M. A. Hamza, W. K. Anderson, *Soil Till. Res.* ***82***, 121-145 (2005).
5. R. Horn, H. Domżżał, A. Słowińska-Jurkiewicz, C. Van Ouwerkerk, *Soil Till. Res.* ***35***, 23-36 (1995).
6. T. Fujikawa, T. Miyazaki, *Soil Sci.* ***170,*** 892-901 (2005).
7. A. Hussain, C. R. Black, I. B. Taylor, J. A. Roberts, *Plant Physiol.* ***121***, 1227-1237 (1999).
8. I. Potocka, J. Szymanowska-Pulka, *Ann. Bot.* ***122,*** 711-723 (2018).
9. F. An *et al., Plant Cell* ***22***, 2384-2401 (2010).
10. D. A. Weits *et al.*, *Nature* ***569***, 714-717 (2019).
11. S. Hartman *et al.*, *Nat. Commun.* ***10***, 4020 (2019).
12. B. Ma *et al.*, *Mol. Plant.* ***6***, 1830-1848 (2013).
13. J. M. Alonso, T. Hirayama, G. Roman, S. Nourizadeh, J. R. Ecker, *Science* ***284***, 2148-2152 (1999).
14. K. D. Montagu, J. P. Conroy, B. J. Atwell, *J. Exp. Bot*. ***52***, 2127-2133 (2001).
15. F. J. Romera, E. Alcantara, M. D. De la Guardia, *Ann. Bot.* ***83***, 51-55 (1999).
16. L. Lamaire, C. Deleu, E. L. Deunff. *J. Exp. Bot.* ***64,*** 2725-2737 (2013).
17. P. Mehra P, B. K. Pandey, L. Verma, J. Giri J, *Plant Cell Environ.* ***42,*** 1167-1179 (2019).

18. W. Zhang, W. Gao, T. Ren, W. R. Whalley, *Geoderma* ***368,*** 114276 (2020).

19. S. R. Tracy, K. R. Daly, C. J. Sturrock, N. M. J. Crout, S. J. Mooney, T. Roose, *Water Resour. Res.* ***51,*** 1006-1022 (2015).

20. G. Huang *et al., Nat. Commun.* ***9,*** 2346 (2018).

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**Competing interests:** Authors declare no competing interests.

**Data and materials availability:** No restrictions are placed on materials, such as materials transfer agreements. Details of all data, code, and materials used in the analysis are available in the main text or the supplementary materials.

**Supplementary Materials**

Materials and Methods

Figs. S1 to S25

Movies S1 and S2

References (16-20)

MDAR Reproducibility Checklist

**Figures:**

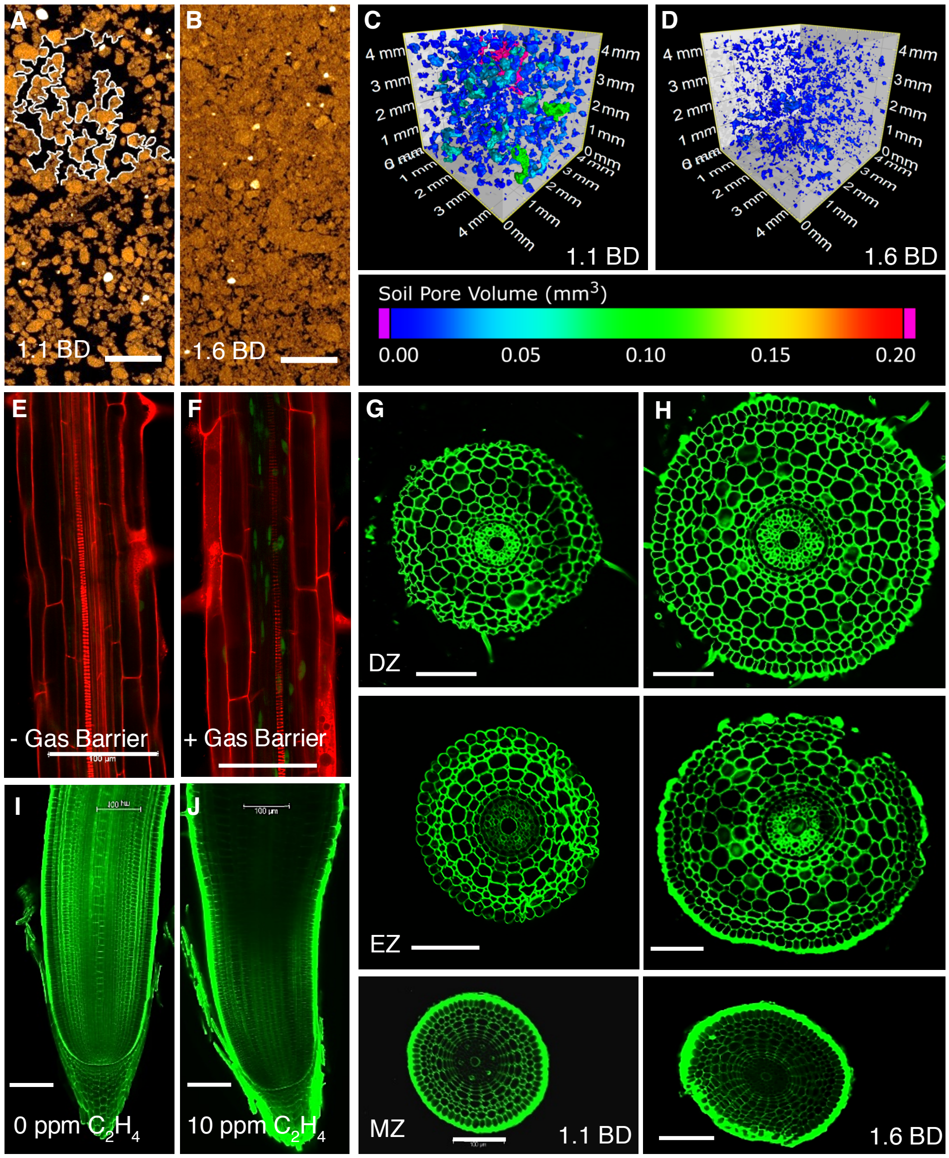


**Fig. 1 Soil compaction reduces the larger pores and triggers root growth responses mimicking ethylene treatment.** (**A** and **B**) CT images showing higher porosity (outlined in white) in uncompacted (1.1 g cm$^{-3}$ bulk density [BD]) (A) versus compacted soil (1.6 BD) (B). (**C** and **D**) Representative 3D images of air-filled soil pores for a 100 x 100 x 100 voxel region from 1.1 BD (C) and 1.6 BD (D) soil cores. (**E** and **F**) Arabidopsis EIN3-GFP reporter exhibits elevated signal after covering root tip with high vacuum silicone grease (+Gas Barrier) for ten hours (F) compared to control (-Gas Barrier) (E). (**G** and **H**) Confocal images of radial cross sections of rice primary roots through meristem (MZ), elongation (EZ) and differentiation (DZ) zones grown in 1.1 BD (G) and 1.6 BD (H) soils. (**I** and **J**) Compared to control roots (I), 10 ppm ethylene treated rice roots exhibit cortical cell expansion (J), mimicking the effect of compacted soil conditions (H). Bars, 1.25 mm in A and B, and 100 µm in G to J.

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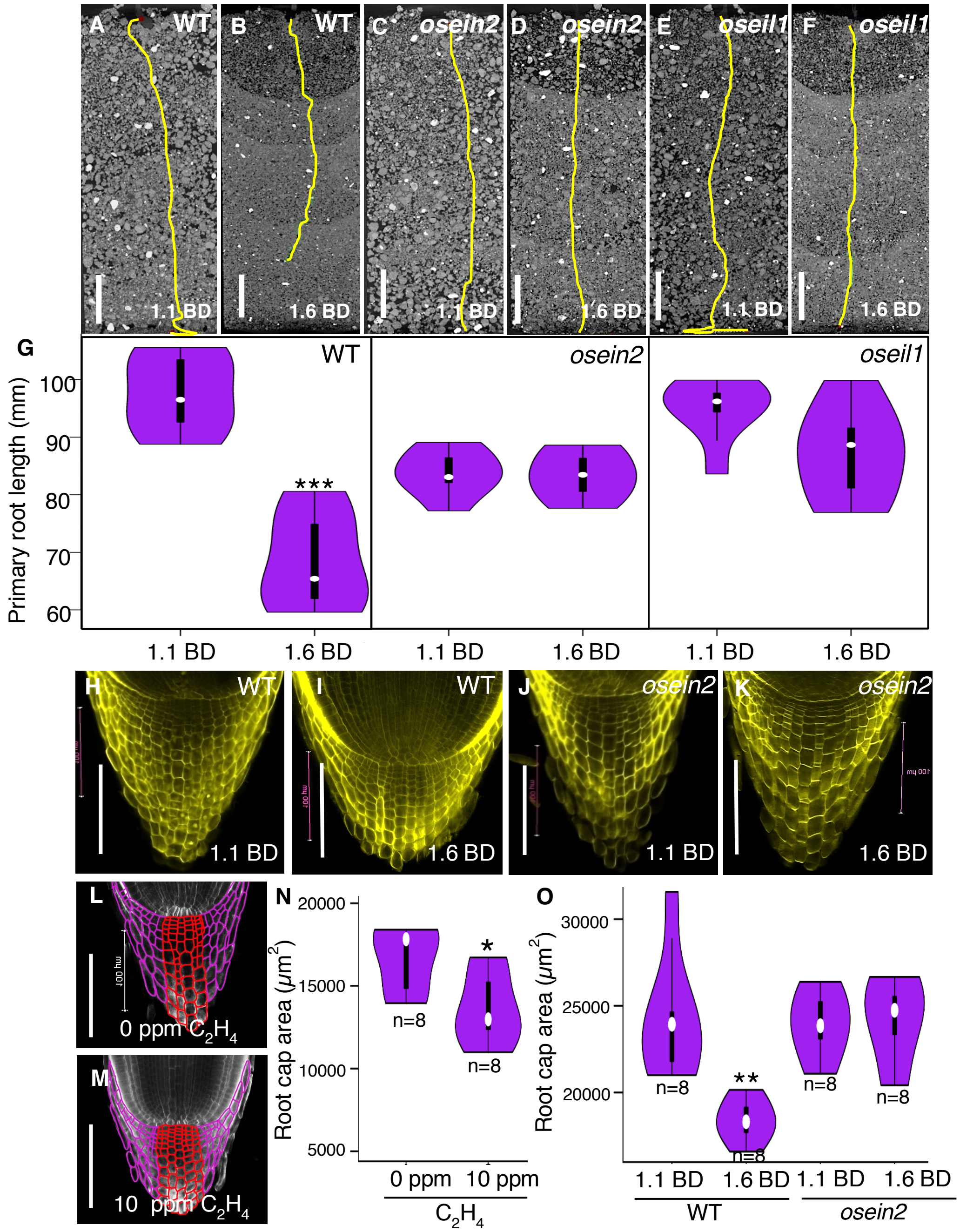


**Fig. 2 Disrupting ethylene response in rice confers root growth resistance to compacted soil.** (**A** to **F**) CT images of primary roots of WT (A and B), *osein2* (C and D) and *oseil1* (E and F) in 1.1 BD (A, C and E) vs 1.6 BD (B, D and F). (**G**) Violin plots of primary root length in uncompacted (1.1 BD) versus compacted (1.6 BD) conditions for WT (wildtype), *osein2* and *oseil1* rice seedlings. (**H** to **K**) Representative images showing root cap area in WT (H and I) and *osein2* (J and K) in 1.1 BD (H and J) vs 1.6 BD (I and K). (**L** and **M**) Ethylene treatment of WT roots showing reduction in root cap area (M versus L). (**N**) Violin plots showing reduction of root cap area after ethylene treatment. (**O**) Violin plots showing reduction of root cap area of WT but not *osein2* when grown in 1.6 BD versus 1.1 BD. Columella cells are marked in red (L and M). *, ** and *** show $p$ value ≤ 0.05, 0.001 and 0.0001, respectively determined using Student's $t$-test. Bars, 10 mm in A to F and 100 μm in H to M.

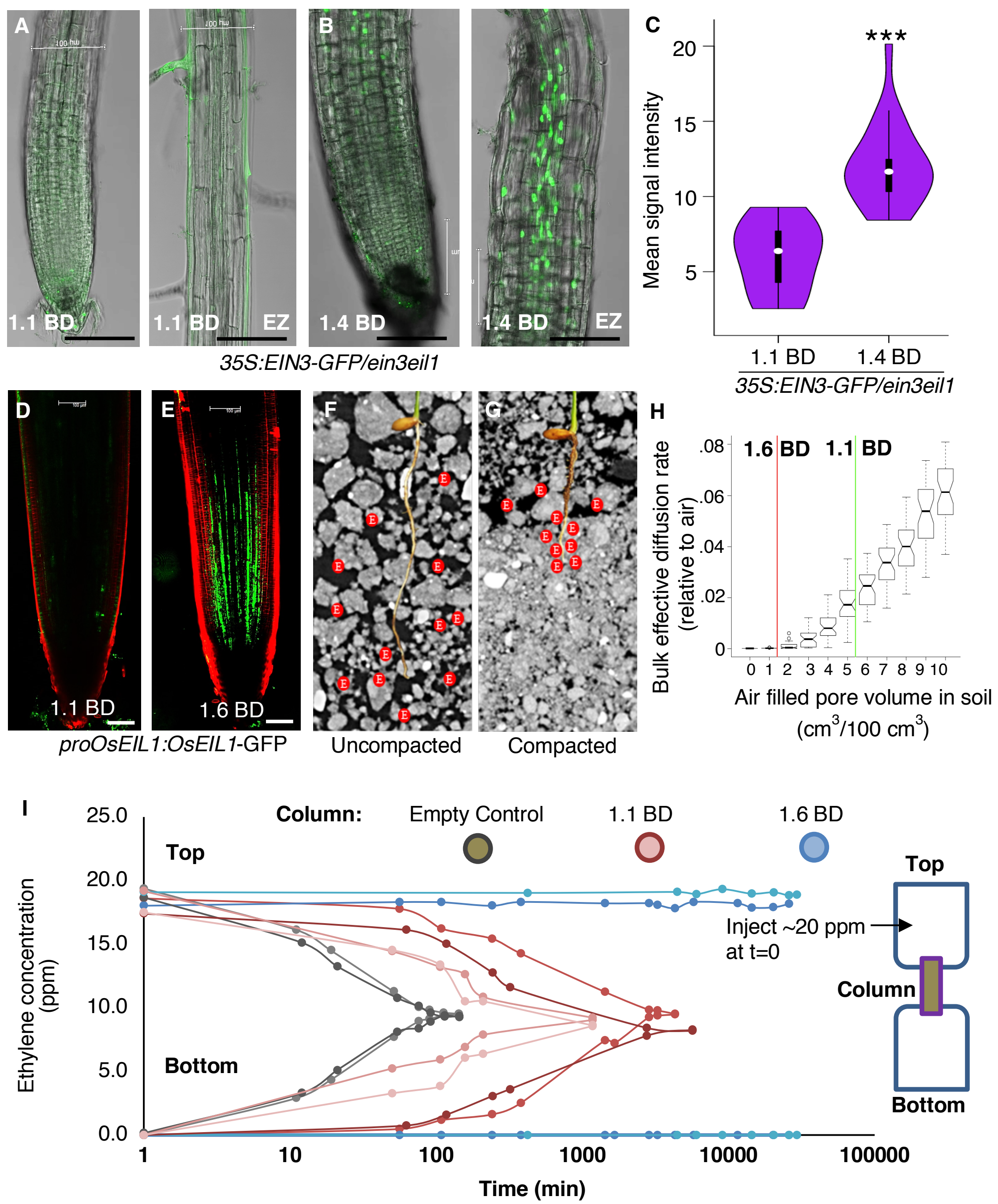


**Fig. 3 Compacted soil reduces ethylene diffusion and enhances root ethylene signalling.** (**A** and **B**) *Arabidopsis* ethylene reporter EIN3-GFP exhibits no nuclear GFP signal when grown in uncompacted soil (1.1 BD) (A), but is clearly detected in root EZ (elongation zone) cells when grown in compacted soil (1.4 BD) (B). (**C**) Violin plot of GFP signal in 1.1 BD versus 1.4 BD in EZ of *35S:EIN3-GFP/ein3eil1*. (**D** and **E**) Compared to 1.1 BD (D) rice OsEIL1-GFP based ethylene translational reporter exhibits elevated signal in compacted soil condition (1.6 BD) (E). (**F** and **G**) Schematic figures of ethylene diffusion (denoted by red circles) in uncompacted (F) versus (G) compacted soil, illustrating preferential accumulation of ethylene around and in root tissues. (**H**) Model simulation showing rate of bulk diffusion of ethylene in soil pores in

uncompacted (green line) and compacted soil (red line). % air equates to $cm^3/100cm^3$ (**I**) Graphical representation of quantification of ethylene across 1.1 BD and 1.6 BD soil layers (1 cm). 20 ppm of ethylene was injected in top chamber. Subsequently, ethylene diffusion in bottom chamber was measured across empty, uncompacted (1.1 BD) and compacted (1.6 BD) soils using GC-MS. *** shows $p \leq 0.0001$ evaluated using Student's $t$-test.