1 Root-soil-microbiome management is key to the success of Regenerative Agriculture

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6 Building soil health and manipulating the soil microbiome, alongside targeted plant 7 breeding that prioritises preferential root architectural development, hold the key to the 8 future success of Regenerative Agriculture. Greater integration is needed between 9 disciplines focused on the rhizosphere scale with plant, microbiome and soil scientists 10 working at the wider farm scale.

11 The management of plant and soil systems for crop production has never been as challenging. 12 Farmers are having to contend with a rapidly changing climate and strong governmental 13 pressures to increase production to satisfy expanding populations. This goal must be achieved 14 while the pace of global urbanisation accelerates, combined with increasing demands to 15 reduce agrochemical interventions and intensive operations which have promoted soil 16 degradation. Against this backdrop, there is renewed interest in conservation-focused 17 farming practices, commonly called 'regenerative agriculture', defined as an alternative 18 means of producing food with lower-or even net positive-environmental and/or social 19 impacts¹. To achieve this demands an interdisciplinary and collaborative research agenda 20 with a specific focus on the management of the root-soil-microbiome interface. Here, we 21 highlight future research priorities that build on current best practice and reveal new areas 22 for translational knowledge exchange.

23 Conflicts of delivering healthy soil

The principles of sustainable soil management associated with regenerative agriculture primarily comprise reducing soil disturbance, building carbon stocks through soil amendments and priming the soil via crop rotation (Figure 1a). In contrast, large-scale cultivation systems have historically prioritised financial gain. Encouragingly, regenerativebased practices that prioritise 'soil health' are now embedded in most government and agriindustry future land-use plans. While exemplars such as utilising cover crops between cash crops have been used extensively, adopting other practices such as reducing tillage has been slower in some regions of the world due to uncertainties around potential yield decline, cost of equipment and concerns that benefits for soil and the wider environment have been oversold.

34 Relatively few studies have compared long-term differences between tilled and no-till soils 35 while adequately controlling potentially confounding variables such as differences in soil type, 36 underlying geology, climate etc. Recent research has demonstrated no-till systems could lead 37 to a reduction in global warming potential of c. 30% (primarily attributed to an increased soil 38 pore connectivity driven by undisturbed soil fauna), with additional benefits for carbon 39 sequestration, which further developed over a time scale > 5 years². Despite this, alternative 40 research has suggested the benefits for soil organic carbon (SOC) under reduced tillage are 41 exaggerated³. A study of 1061 pairs of tilled versus no-till soils and concluded SOC only 42 increased in the 0-10 cm soil layers under no-till and decreased at depth, leading to an overall 43 reduction⁴. However, this has since been rebutted in another study after analysis of the same 44 dataset which found no-till led to preferential SOC storage⁵, highlighting the conjecture in this 45 area.

46 **Opportunities for plant breeding**

47 Rapid changes in soil management practices adopted by farmers such as no-till represent a 48 major challenge for crop breeders, given the urgent need to select new varieties better 49 adapted for 'regenerative agriculture' approaches and a changing climate. For example, 50 reducing tillage results in a harder topsoil, especially near the surface, though this may 51 dissipate over time².

A future hope is that breeders could select new crop varieties adapted to deal with enhanced compaction resistance and thus counteract harder soil. Selecting crops with roots exhibiting improved compaction resistance is a very labour-intensive process given the challenges of field root phenotyping⁶. However, the recent identification that the plant hormone ethylene controls root responses to hard soil opens new opportunities to rapidly select compactionresistant crops⁷; especially important as soil compaction is widespread. To date, root growth sensitivity to ethylene appears to be a good proxy for sensitivity to compaction stress in important cereal crops such as maize⁶ and rice⁷. This provides breeders with a new, highthroughput phenotyping method to rapidly select new crop varieties with improved compaction resistance, likely to be a key requirement for growth in the soils of the future.

62 Climate change is having an increasing impact on soil moisture. As topsoil dries, a vertical 63 gradient in water availability forms. Roots experiencing water deficit increase their angle to 64 better access deeper soil profiles⁸. Reduced moisture in the topsoil leads to suppression of 65 lateral and crown root growth in many crop species. A crop ideotype with fewer but longer 66 laterals could be more efficient during water stress, ensuring valuable plant resources are 67 used to extend the root system into the subsoil. Similarly, a crop ideotype with a steeper root angle would support water acquisition in subsoils, especially if there were more biopores 68 69 (Figure 1b&c). Several regulatory genes have been identified in major cereal crops that 70 control root angle⁸. However, root colonization of subsoils is often challenging due to 71 increased mechanical impedance created by overburden pressure. Cereal roots, such as 72 wheat, growing at depth (e.g. >50 cm) are predominantly found in soil biopores⁹ (Figure 1b). 73 Maize and soybean roots can preferentially grow towards vertical biopores using an adaptive 74 process called *trematotropism*¹⁰. Discovering the mechanism of how roots respond to overburden pressure is crucial for future selection of deeper rooting varieties adapting to soils 75 76 under climate change.

77 A steeper root angle also promises to improve the sustainability of crops. For example, nitrate 78 is a challenge for roots to capture due to its high mobility, causing it to leach deeper into the 79 soil profile. Breeders could exploit steeper root angle for more efficient capture of nitrate 80 (Figure 1b). Alternatively, plants that proliferate fine roots in the topsoil can reduce nitrate 81 losses via the establishment of micropores where water/solute retention is enhanced¹¹. 82 Conversely, phosphate is a highly immobile macronutrient, often concentrated in the topsoil. 83 To improve the capture of phosphate (and reduce the need to apply this non-renewable 84 macronutrient), breeders could select ideotypes with shallower root angles (Figure 1b), an approach which has worked very well for beans¹². A key regulatory gene through which 85

phosphate limitation controls root angle has been identified in rice¹³, opening new
 opportunities to develop novel varieties with adaptive traits for future soil environments.

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Manipulation of the soil microbiome

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Soil-grown plants are colonised by a wide range of taxonomically diverse soil microbial communities which establish both beneficial and detrimental associations with plants that impact their growth and fitness across different ecological contexts, including agricultural settings. Hence, plant microbiota and soil health are inextricably linked through a circular regulatory mechanism in which factors impacting on soil health also alter properties of beneficial plant-associated microbiota and vice versa (Figure 1d).

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97 Disease-suppressive soils are a clear example of the soil-plant connection as they protect 98 plants against infections by soil root pathogens offering great potential to increase 99 agricultural sustainability. Future research must take advantage of advances in culture-100 independent technologies to study soil microbiomes, such as metagenomics, to reveal the 101 ecological and molecular mechanisms behind pathogen suppression and facilitate the use of 102 disease suppressive soils. For example, metagenomics can identify the bacterial taxa and 103 functions responsible for the different layers of defence against pathogens in the rhizosphere 104 and root endophytic compartment, respectively¹⁴.

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106 Understanding microbial community dynamics, metabolic interactions among microbial 107 community members, and microbial characteristics associated with pathogen suppression 108 will help to predict if soils will develop pathogen suppressive characteristics supporting future 109 crop production. However, to fully leverage microbial advantages, we need to increase efforts 110 to define and understand the co-evolutionary processes governing the assembly of the plant 111 microbiota. This will allow us to optimise the interaction of plants with beneficial microbes, and to use the advantages of the microbiota efficiently and reproducibly in plant health 112 113 conferring subsequent benefits for soil health.

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115 Effective management of the root-soil-microbiome

116 Sustainable approaches for soil management that maximise carbon inputs and facilitate a 117 naturally-derived soil structure, closely integrated with crop breeding programmes that target 118 root adaptive traits to thrive under changing conditions are essential. At the heart of this is 119 an urgent requirement to develop a greater understanding about how to manage the root-120 soil-microbiome most effectively in regenerative agri-systems. In addition, greater 121 engagement between plant (shoots as well as root specialists) and microbiome researchers 122 with soil scientists, crop breeders and farmers is vital to achieve this urgent objective. For 123 example, the association between plant genotypes, root and shoot microbiota composition, 124 and the molecular mechanisms underpinning plant-microbiota interactions could inform 125 breeding to optimize crops in response to environmental fluctuations such as those anticipated in the soils of the future^{15,16}. 126

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The effective engagement outlined here requires imaginative public and/or private funding initiatives to promote inter-disciplinary interactions between researchers and commercial partners beyond that at present. Without such urgent intervention, the ability to translate new research findings to underpin and enhance the enormous potential impact of regenerative agriculture will be severely limited.

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- 181 Figure 1: Multiscale representation of how the principles of regenerative agriculture might
- 182 be supported by new efforts in plant breeding and manipulation of the root-soil-
- 183 *microbiome to enhance food security.* (a) Field Scale: Integrated crop-livestock system
- 184 where manures and residues are left on the soil surface under a no-till approach; Soil Profile
- 185 Scale: b) Under long term (>10 years) no-till, a thriving soil faunal community creates a
- 186 network of extended biopores facilitating deeper root penetration to access water and N
- 187 where extended biomass in the topsoil enhances P acquisition, in comparison to c)
- 188 conventional tillage where a plough pan and reduced faunal activity limit deeper rooting
- 189 especially in the subsoil and (d) Root Scale: manipulation of the soil microbiome in the
- 190 rhizosphere create an oxic environment supporting enhanced water and nutrient use
- 191 *efficiency and disease resilience due to root-microbe interactions.*

192 Competing interests

193 The authors declare no competing interests.