

Review of applying X-ray computed tomography for imaging soil-root physical and biological processes

Francis Kumi^{1,2}, Mao Hanping^{1*}, Hu Jianping¹, Ikram Ullah¹

(1. Key Laboratory of Modern Agricultural Equipment and Technology, Ministry of Education and Jiangsu Province, Jiangsu University, Zhenjiang 212013, China; 2. Department of Agricultural Engineering, University of Cape Coast, Cape Coast, Ghana)

Abstract: Root growth process in soils has long been a matter of interest to soil and plant scientists. However the opaque nature of the soil has been a barrier to most research attempts aimed at unraveling the full root-soil processes. The traditional method of separating the roots from the growth media which is common practice tends to be destructive and defeats the purpose of such studies. It is ineffective in monitoring the interactions within the soil medium and as a result, a fast non-destructive technique is preferred. However, with computing and technological advancements, X-ray computed tomography (CT) has been found to be capable of meeting this need by imaging the processes which are of interest to researchers. Over the past three-four decades, the applications of the technology for imaging soil-root studies have attracted the researchers' widespread interests and the future looks more promising. The purpose of this review is to present an overview of CT applications in imaging root-soil processes. The main focus is on the use of soil-root interface researches and the way forward for such non-destructive analyses.

Keywords: computed tomography, soil, root, imaging, non-destructive, interactions

DOI: 10.3965/j.ijabe.20150805.1490

Citation: Kumi F, Mao H P, Hu J P, Ullah I. Review of applying X-ray computed tomography for imaging soil-root physical and biological processes. Int J Agric & Biol Eng, 2015; 8(5): 1–14.

1 Introduction

The soil is a heterogeneous system with biotic, abiotic and structural interactions^[1]. Paramount among these is the kind of interactions existing between the root and its environment^[2]. A casual evaluation of research over the

past years reveals the need to fully understand the processes within the full soil medium and the mystery surrounding the interactive mechanisms such as root growth and soil microstructural changes^[3]. The spatial response of the root to soil environmental conditions in the face of changing climatic features plays a critical role in understanding the dynamics of such systems^[4].

Traditional methods for studying root architecture and morphology have been destructive in nature, making it difficult for comprehensive soil-root interactive analysis. To overcome this limitation, non-destructive imaging tools such as Thermal Neutron Tomography (TNT), Magnetic Resonance Imaging (MRI) and X-ray computed tomography (CT) have been used by researchers^[5-8] to investigate the soil natural processes. The development of such high-resolution imaging technologies has paved the way for visualization and quantification of the morphological changes with sufficiently accurate configurations. However, the commonly utilized

Received date: 2014-10-12 **Accepted date:** 2015-10-02

Biographies: Francis Kumi, PhD Candidate, Research interest: vegetable transplanting root growth, development and transplanting damaging mechanisms using X-ray computed tomography. Email: fekkumi@yahoo.com; Hu Jianping, PhD, Professor, Research interest: design of transplanters. Email: hujp@ujs.edu.cn; Ikram Ullah, PhD candidate, Research interests: X-ray computed tomography and tomato growth. Email: ikram2155@gmail.com. *Corresponding author: Mao Hanping, PhD, Professor, Research interest: monitoring vegetable root development, transplanting and greenhouse technologies. Address: 301 Xuefu Road, Key Laboratory of Modern Agricultural Equipment and Technology, Ministry of Education and Jiangsu Province, Jiangsu University, Zhenjiang 212013, China. Email: maohp@ujs.edu.cn, Tel: +86-13511695868.

options are MRI and CT. Pohlmeier et al.^[9] observed that although MRI is useful in monitoring water content dynamic changes in the soil-root system, it becomes more effective when it is combined with other multiple-point techniques in fine-grained soils. The content of an overview by Moradi et al.^[10] pointed out how MRI has been applied in imaging soil-water-root systems even though the presence of magnetic particles in the soil limits the production of quality images compared to CT. Further, the former technology is more costly, hence not widely available to researchers. Additionally, it requires relatively longer period of time to scan the samples for analysis with low resolution^[11-13]. Consequently, Helliwel et al.^[8] published a comprehensive review on the application of CT to evaluate soil biophysical interactions and structural development. They approved it as an indispensable tool for characterizing systemic processes in the soil.

In recent times, there have been a considerable number of research attempts aimed at quantifying complex three-dimensional (3D) root structure architecture in soils using CT. Such progress is important for better appreciation of the role of root growth in crop production^[14]. This review gives the recent progress in the use of CT especially for 3D investigations related to soil-root interactions research. We provide brief overview of the technical and theoretical fundamentals of CT. The main content discusses the current trends in root-soil interface research: physical and biological processes. Of particular interest related is the focus on the past and current investigations and the possible recommendations for further research.

2 Overview and theory of CT

The invention of CT is largely attributed to the work of the 1979 Nobel prize winner, Godfrey N. Hounsfield in 1973^[15]. Although the original purpose was for medical diagnostic investigations, Petrovic et al.^[16] were among the first to have used it to assess soil bulk densities. Consequently, Hainsworth and Aylmore^[17] utilized it to investigate the spatial distribution of water uptake by roots. Subsequent interest in CT use in soil research led to rapid interests in the visualization of soil

dynamic conditions among researchers^[9,18-22]. In the initial stages, some were quite skeptical about its potential negative influence on soil biological properties and organisms but many works have reported contrary views to this^[23,24]. It has thus extensively been used in a wide range of applications^[25]: root network and architecture^[26,27], soil compaction^[28,29], soil physical properties^[30,31], soil hydraulic characteristics^[32,33] and root-soil dynamics^[8,23,34,35].

2.1 Basic principle of operation

Although the technology has undergone several improvements, its working principle and functionality remain the same^[36]. This has been extensively reported by Wildenschild^[37], Stock^[38], Taina et al.^[39], Mooney et al.^[36] and Helliwel et al.^[8], hence a short overview on the principle of operation is covered in this section.

The visualization of the interior elements of an opaque sample is made possible by the principle of electromagnetic wave attenuation. A typical scan system consists of three main components: a source of X-rays, a platform for placing the sample under investigation and the detector being placed at the receiving end. The X-rays from the source then move through the object under investigation which absorb and scatter the photons^[36]. It is positioned in between an X-ray source and X-ray detector^[40]. The process of randomly reconstructing the distribution of a physical parameter from different angle positions is called tomography^[41]. The decrease in intensity of X-ray moving through the sample under investigation is also termed attenuation^[36]. The X-ray source could be a cone-beam or a synchrotron type. With the cone-beam type (often called the medical CT), one can choose an appropriate magnification of the object in the radiography: the closer to the source, the higher the magnification and thus the higher the spatial resolution. In most cases, the sample could be rotated closer or away from either the source or detector for change in spatial resolution and magnification^[42]. It is very important that the sample is firmly fixed in its holder to avoid any relative movement between the two during scanning (Figure 1).

On the other hand, synchrotron scanners use light

generally designated to emit electromagnetic radiation of electrons moving at super-high-velocities (close to the speed of light). It is then forced to change direction in order to interact with the path of the electrons under the action of a magnetic field^[35,44]. The radiated energy is proportional to the fourth power of the particle speed and is inversely proportional to the square of the radius of the path. The reader is referred to Aravena^[29] for further and detailed description on the principles and applications of synchrotron X-ray imaging.

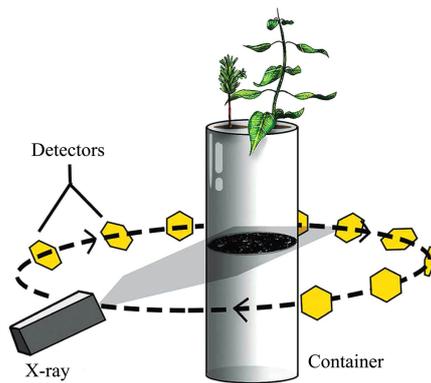


Figure 1 Illustration of the principle of CT in imaging slices of plant soil^[43]

The spatial resolution of the images depends on factors such as the magnification, focal spot size of the X-ray tube, pixel size of the detector and other physical factors such as X-ray scattering and interaction among detector pixels. The attenuation coefficient of the material under investigation to X-ray also depicts its density. Thus the intensity is expressed as:

$$I = I_0 \exp(-\mu_m \rho x) \quad (1)$$

where, x is the penetrating length of incident X-ray; ρ is the density of material; μ_m is the absorbing coefficient per unit mass of detected object; I is the intensity of X-ray after penetrating object; I_0 is the intensity of X-ray before penetrating object^[45]. In its operation, each scan obtained may contain an array of pixels which describe the attenuation coefficient of the object expressed in Hounsfield units (HU) which describes the attenuation of the voxels in a 3D scan. The tomographic numbers, represented as HU with a linear attenuation coefficient (μ), is expressed as:

$$HU = 1000(\mu - \mu_w) / (\mu_w - \mu_a) \quad (2)$$

where, μ_w and μ_a are the attenuation coefficient of the water and air, respectively^[39].

2.2 Image segmentation and analysis

Image segmentation is used to assign CT numbers^[46]. This is a critical stage involving the separation of components according to the attenuation density, with the darker voxel having low attenuation while that of the lighter voxel have high attenuation. The inability to carefully remove the focus of investigation from the other non-essential parts of the sample (by way of using the contouring feature in the system) could have a consequential negative impact on the results. Using CT in conjunction with specific software enables researchers to correctly visualize and quantify different spatial distributions in soils^[47]. In their comprehensive review, Helliwell et al.^[8] stated segmentation approaches in soil research: global and dynamic thresholdings. The former is based on estimation from the image's histogram while that of the dynamic type has different values applied to different segments of the image's region. However, it was not clearly stated as to the recommended thresholding range that should be adhered to. With respect to soil-root studies, De Smet^[48] suggested that time is essential in considering such analyses of root systems in soils.

3 Application of CT for soil-root research

In the 1980s Tollner and colleagues^[49] successfully used X-ray imaging to monitor biotic and abiotic interaction within the soil medium. It was subsequently used for detecting the soil's chemical granule incorporation and distribution^[50], predicting water flow^[51] and estimating bulk density^[52] as well as measuring particle sizes^[53]. In an attempt to minimize the errors associated with the use of CT imaging to measure soil physical properties, Tollner et al.^[54] proposed a method for estimating CT scans. The technology has proven to be one of the most promising modern techniques for soil-root processes^[17-19,26,39,55-60]. Over the past three-four decades, visualization using CT has seen rapid advancement with higher and quality image resolutions. It has been applied in extensive areas of soil research including root-soil structure^[20,23,34,57,61,62], organic matter content^[63,64], hydrological dynamics^[7,21,65-67], deformations^[68] and tillage systems^[60]. Studies in relation to the interactions between the biotic and physical environmental

factors under varying dynamic conditions over time which is hidden from the naked eye has as well been reported^[48]. With rapid interest in using CT for visualizing and quantifying roots, it is now recognized as an accepted tool in soil and plant science communities with the potential of examining root system architecture

and the establishment of the relationship between physical and biological properties. Table 1 shows a summary of the key research papers in this field over the past decade. Further discussion on the physical and biological processes between the roots and soil are presented.

Table 1 Summary of key works on the use of CT in soil-root research in the past decade

| Author | Year | Focus of research | 2D/3D | Area(s) for further research |
|-----------------------------------|------|---|-------|---|
| Mooney et al. ^[69] | 2006 | Visualizing root lodging in soils | 2D | Distinct root lodging mechanism for different crops |
| Kaestner ^[26] | 2006 | Root network visualization | 3D | Models to predict root performance in soils |
| Feeney et al. ^[70] | 2006 | Microbe-root impact on soil structure | 3D | Microbe biotic range of activities in the soil acceptable for root growth |
| Lontoc-Roy et al. ^[71] | 2006 | Visualization of maize roots | 3D | Root morphology analysis in relation to soil structure |
| Perret et al. ^[20] | 2007 | Root/root tip of imaging chickpea | 3D | Root dynamic mechanisms in the rhizosphere |
| Hamza et al. ^[72] | 2007 | Osmotic potential effect on roots | 2D | In-situ CT evaluation of plant roots |
| Han et al. ^[73] | 2009 | CT assessment of pathogens on roots | 2D | CT scanning of soil-root phytopathology |
| Hargreaves et al. ^[74] | 2009 | Analysis of root characteristics | 3D | High-resolution in-situ imaging of roots |
| Carminati et al. ^[61] | 2009 | In-situ imaging of root-soil gaps and interactions | 3D | Relationship between root water uptake and gap location |
| Seigneur et al. ^[75] | 2010 | Characterization of roots grown in pollutes soils | 3D | Roots' spatial distribution |
| Bogart et al. ^[76] | 2010 | Corm-tissue morphology and specie | 3D | Physical and chemical micro-structure dynamics of corm tissues |
| Tracy et al. ^[27] | 2010 | Assessment of root architecture in undisturbed soils | 3D | Soil-root interactions under different soil structures |
| Aravena et al. ^[77] | 2011 | Root-induced compaction effect on water properties in the rhizosphere | 2D | Root growth mechanisms and water uptake characteristics |
| Garbout et al. ^[78] | 2012 | Soil and root structure | 2D | Abiotic stresses effect on soil-plant interactions |
| Tracy et al. ^[79] | 2012 | Impact of compaction on root growth | 3D | Modeling root functional architecture in different soils |
| Mairhofer et al. | 2012 | Plant root architecture | 3D | Root phenotyping as basis for crop breeding |
| Schmidt et al. ^[80] | 2012 | Root extraction evaluation methods | 3D | Combination of mathematical soil and root modeling and magnetic resonance imaging |
| Kopittke et al. ^[81] | 2012 | Arsenic effect in cowpea root growth | 3D | Root border cells' role in arsenic osmotic transport |
| Mairhofer et al. ^[82] | 2013 | Root architecture recovery from soil | 3D | Characterization of plagiotropic root architecture |
| Zappala et al. ^[83] | 2013 | Influence of X-ray dose on root growth and soil microbial population | 3D | Impact of X-ray doses on root-soil properties (continuous research) |
| Zappala et al. ^[84] | 2013 | Root segmentation from soil as influenced by moisture content | 3D | Scanning errors consideration in root segmentation in soil |
| Carminati ^[85] | 2013 | Soil gaps as influenced by water potential and transpiration | 3D | - |
| Aravena et al. ^[86] | 2014 | Mechanical deformations from root growth and its effect on rhizosphere water uptake | 2D | Modelling the impact o compaction on root growth in different soils |
| Koebnick et al. ^[87] | 2014 | Dynamics of root growth in soils | 3D | Water and nutrient uptake models on the bases of root spatial arrangements |
| Tracy et al. ^[88] | 2015 | Impact of compaction on roots | 3D | Dynamic Root-soil mechanism |
| Mairhofer et al. ^[89] | 2015 | Root extraction evaluation methods | 3D | Combination of mathematical modeling and magnetic resonance imaging for roo-soil analysis |
| Daly et al. ^[90] | 2015 | Visualization of water distribution in bulk and rhizosphere soils | 3D | Water uptake models based on rhizosphere biophysics |
| Paya et al. ^[43] | 2015 | Root growth intra-and-interaction with undisturbed soil space | 3D | Root sensitivity and response to roots in neighboring crops |
| Ahmed et al. ^[91] | 2015 | Root interaction with fertilizer granules | 3D | Optimization of placement and granule size in relations to crop life cycle |

Note: Gap indicate unreported information in literature.

3.1 Soil-root physical processes

3.1.1 Root architecture imaging

Mooney et al.^[69] in 2006 were among the first to have utilized CT to study lodging roots in soils. The authors

visualized the rice roots, although the full mechanism of the root-soil could not be clearly explained. Consequently, Kaestner et al.^[26] presented one of the best root architecture systems using CT. Four months old

alders (*Alnus incana*. L Moench) were re-grown in an artificial sandy soil and scanned at a CT resolution of 36 μm for 8.7 h. This resulted in clear imaging of fine root including those which are less than 0.5 mm, suggesting success in their procedure. Similarly, Lontoc-Roy et al.^[71] utilized high-resolution CT scanner to produce 500 images from 5 d old maize seedlings grown in predominantly homogenous sand and sieved loamy sand soils. The authors reiterated the need to dry soils prior to CT scanning as a way of obtaining quality scans. They proposed using non-homogenous soils in future investigations.

In 2007, key works by Perret et al.^[20] and Hamza et al.^[72] made significant contributions to CT root imaging research. Perret et al.^[20] developed a protocol to quantify chickpea (*Cicer arietinum*) roots after 21 d of growth. Some of the major parameters obtained in the work include volume, wall surface area, length, tortuosity, orientation and number of roots using high resolution CT. Destructive procedure was similarly used to evaluate the physical parameters of the root architecture and compared with the results from the non-destructive analysis. Consequently, the authors noted the usefulness of CT in investigating root system architecture, although they acknowledged that at the time, their work could not be as effective as destructive methods especially in clearly obtaining the full length of roots. They also proposed the use of their algorithm for a more in-depth analysis of the root-soil mechanisms in the rhizosphere. Hamza et al.^[72] then used CT to investigate the influence of salt stress on the root growth of lupin (*Lupinus angustifolius* L.) and radish (*Raphanus sativus* L.) in the range of -2.0 MPa to -0.10 MPa. There were significant differences in the responses of the selected crops to the osmotic stresses. The lupin roots could not withstand low stress concentration of -2.0 MPa while the radish was more responsive in recovering from this low stress after the removal of the treatment.

In the subsequent years, Han et al.^[73] and Hargreaves et al.^[74] published interesting reports. Upon successfully extracting the roots of potato, Han et al.^[73] further investigated the effect of density on the common scab-inducing bacterium on the growth of the roots.

They assessed the underground part of the stem and the first-order roots of the crop. In their experimentation, two groups of plant: diseased plants inoculated with a bacterium (*S. scabies* EF-35) and that of healthy crops, were used. The investigators encouraged further research in the use of their protocol for assessing phytopathological plants. Further approach by Hargreaves et al.^[74] demonstrated the capability of overcoming limitations associated with root extraction from soil systems. The study was based on the characterization of barely seedling roots using gel chambers and soil sacs embedded in pots of soils for CT imaging. They were successful in using CT data to model root architecture. Mairhoffer et al.^[82] also introduced a complete method using CT at a resolution of 23.91 μm for the recovery of tomato (*Solanum lycopersicum* L.) roots grown at for 10 d using RooTrak as a segmentation tool (Figure 2). In another study Mairhofer et al.^[89] focused on straightening out issues concerning the appropriate ways of segmenting roots from natural soils by comparing three different methods. The authors compared different segmentation tools used for evaluating CT images of roots in soils. VGStudio Max and RooTrak, among other tools are effective in recovering roots from the soil from CT scans. RooTrak has been used in tracking several lateral and main roots of different crops and has proven to be distinct in its application^[14]. Another step towards making the roots recovery and quantification caused Karunakaran et al.^[92] to consider using X-ray absorption and phase contrast imaging techniques for effective quantification of plant roots in the soil under real-time conditions. The soil types and the scanner's resolution, among other factors, were reported to be important parameters required for producing high quality images of roots.

Very recently, research works from Paya et al.^[43] and Ahmed et al.^[91] have enhanced the potential of CT as a tool for the spatial in-situ examination of roots. Some of these recent researches now utilize CT to address very interesting research questions regarding the mechanisms at the root-soil interface. While Ahmed et al.^[91] focused on the use of 4D μCT to assess level of root-organic matter interaction at the micro-level, Paya et al.^[43] went a

step further to investigate the sensitivity of one root from the nearby roots. The latter authors reiterated the potential of using high spatial resolution μ CT for providing 3D structural which could help determine the process of modeling the interactions between roots of plants.

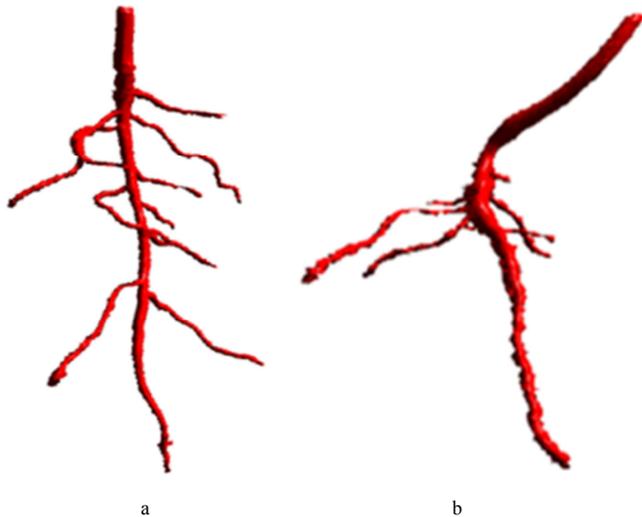


Figure 2 3D visualization and extraction by RooTrak for tomato seedling grown in clay loam (a) and loamy sand (b)^[82]

3.1.2 Soil compaction and structure deformations

The management of soil structure is essential for the growth and stability of plants. Soil structural changes involve particle re-arrangement as well as reduction in specific volume and increase in bulk densities^[93,94]. Root growth also contributes to the displacement of soil particles causing cracks, voids and pores. Although it is generally accepted that changes in the structure of the soil (e.g. compaction) inhibits the growth and development of roots^[94], recent report from Aravena et al.^[77] reiterated that a little form of root-induced soil compaction is beneficial to the overall growth and development of the crop.

In their earlier investigations, Petrovic et al.^[16] utilized tomographic scans to evaluate the bulk densities of soils. The need to investigate the quantification of soil structure by establishing the relationship between the pores and roots was reiterated by Jassogne et al.^[95] In monitoring the effect of the clay-cation bond on soil structure, Marchuk et al.^[96] noted that structural changes during soil–water interaction is dependent on the bonding properties of its clay–cation contents. Soils saturated with water prior to each CT scanning enhances the

root–soil density contrast and enables the facility to conveniently distinguish roots from the rest of the soil medium^[97]. Recent study by Tracy et al.^[98] considered growing abscisic acid (ABA) endowed tomato genotype and another deficient in ABA for assessing the level influence of this crop root induced chemical to remediate the negative impact of compaction on the growth and morphology of roots. Root response mechanism to changes in the soil is still not clear and as result, the authors tried to find ways of meeting this challenge. The findings indicated that except for tortuosity, all other parameters measured and analyzed in the work showed that wild type genotype increased more than *notabilis* under the soil compaction levels. The study concluded that the presence of ABA reduces the negative effects of compaction on the growth and development of root and favors crop growth eventually. It mediates and helps in the improvement of the morphological characteristics of the roots. The limitation of the study was that it was done under a single soil type and moisture content. Despite these attempts, there is still lack of full understanding into the progressive mechanism of such soil dynamic conditions. Recent study on the effect of stress on frozen soil using μ CT carried out by Bhreasail et al.^[68] could be used as a benchmark for assessing the structure and deformation mechanism in soils.

3.1.3 Soil- roots hydraulic interactions

There is a general increasing interest in imaging roots and the soil water uptake concurrently with its effect on the surrounding media so as to explore how roots behave under different environmental conditions^[99]. Accordingly, root system visualization using low resolutions has been the central theme for numerous studies looking at plant, soil, water and nutrient interactions^[100]. However, despite the difficulty in segregating the moisture attenuation from other soil components, some assumptions are generally made in the estimation of such parameters under uniform compaction level^[101]. The parameters and analytical procedures used for CT scanning obviously need proper adjustment for monitoring the effect of watering and other processes in the soil medium^[102].

In recent years, however, the use of CT to monitor

soil water flow properties is a rapidly evolving research area. Carminati et al.^[85] noticed that ‘gaps’ in soil structures (under soil-lupin root interactions) are principally caused by reduction in the availability of moisture and found the effect even to be more pronounced around the lateral roots. Brodersen et al.^[103] provided an excellent commentary on the use of non-invasive methods for imaging dynamics in the soil as a result of root water uptake and transportation. The main limitation reported by the authors, is the inability of CTs to clearly monitor three-dimensionally, the in-situ flow properties of the water. With improvement in the resolution, numerical modelling and the use of appropriate contrast agents in experimentation, more in-depth analysis can be performed in soil and plant research. Consequently, Aravena et al.^[86] used numerical modeling and CT to elucidate the effect of hydraulic flow properties on the root axial expansion in the rhizosphere. The authors found that an increase in compaction among soil particles in this region tends to improve its water flow properties. Hence, the bigger the radius of the root, the more likely it is to positively influence its hydraulic uptake properties. However since the study was performed under low densities, future investigations into the deformation dynamics under compacted soils could help in deepening our understanding of these mechanisms. Dal Ferro^[104] attempted to model the hydraulic conductivity of sandy loam cambisols and noticed that the quantitative determination of pore size is interlinked with the resolution and type of soil in meeting the set objective. Further research into the root-soil contact by way of finding out the hydraulic properties of the soil is imperative across a range of soil types. Very recent reports have begun showing how a number of such bottlenecks could be overcome. Excellent studies by Subramanian et al.^[105] utilized CT, to evaluate complex below-ground features of maize seedlings under different water stress conditions within a period of three weeks after sowing. The drawback to the study was the relatively short duration of the experiments, and as result, the effective stress mechanism of the crop in its overall life-span could not be reported. The authors proposed

further research in this direction.

3.2 Soil-root biological processes

Modelling root systems presents an opportunity to investigate functional tradeoffs between foraging strategies (i.e. shallow vs deep rooting) for contrasting resources (immobile versus mobile resources), and their dependence on soil type, rainfall and other environmental conditions. Such models are often useful foundation for exploration, interpretation and subsequent validation of experimental results^[48]. The first root model was established by Lungley^[106] and later work by Dunbabin et al.^[4] have stressed the need to couple such models with another model representing the soil environmental conditions.

CT offers an effective non-destructive approach for the quantification and physical analysis of biological activities in the soil^[107]. For maize seedlings grown in homogeneous sand, for example, it has been found that the dry condition provided a more complete 3D representation of the root system than the water-saturated condition. Hence, the integration of high-resolution CT scanning and computer programming allow for 3D visualization of crop root systems in appropriate soil-moisture combinations (e.g. dry homogeneous sand, water-saturated loamy sand^[102]). Pierret et al.^[99] studied root growth by taking the differences between the initial stage of root imaging after transplanting and that of the later image to vividly describe the root development over time using X-ray transmission imaging system. It was obvious from their study that using suitable X-rays imaging techniques, numerous soil-root related issues could readily be addressed.

Soil scientists basically describe the soil as being composed of the bulk soil and the rhizosphere. The rhizosphere is the area close to the root where multiple dynamic activities take place^[108] and the rest being the bulk soil. The term, rhizosphere, was introduced by Hiltner in 1904 to describe the critical environmental interface close to living roots which regulates the transport of solutes, nutrients, gas exchange and water supply from the soil to the plants^[29,109]. A healthy rhizosphere population can help plants deal with stresses experienced by the root^[110]. In the past, a number of

researchers have often focused on bulk soil studies to the neglect of the occurrences in the rhizosphere^[111]. However, recent reports by Darrah^[112], Dunbabin et al.^[113], Dupuy et al.^[114] and Zappala et al.^[83] showed fascinating findings in this minute aspect of the soil structure. Seignez et al.^[47] demonstrated that CT technique could be used to obtain a better understanding of the behavior of plant root-soil under different levels of physical and chemical polluted compositions. The authors characterized and performed CT's 3D quantification to reveal the growth of hyper-accumulative roots in such soils. This buttresses the point that coupled with specific softwares, CT has the potential for measuring and quantifying most physical and structural properties in the soil^[115].

The physical, biological and chemical structures of soil are inherently influenced by the activities of microbes, earthworms and insects^[116-119]. Their interactions with the soil among other factors contribute to complexity in such media. Studies related to the understanding of the dynamics cannot fully come to the fore without having full understanding into the behavior and configuration of such processes. Until recently, there have been difficulties in the in-situ studying of the soil microbial habitats and their operations. Nonetheless, amidst challenges, researchers have investigated such activities and described their influence on the soil as self-organizing in nature^[120]. One of the main issues confronting the use of CT in such investigations, however, is the difficulty in distinguishing between the attenuation of natural soil mineral particle arrangements from that caused by soil microbes.

CT was used by Zappala et al.^[83] for the 3D visualization and quantification of microbial population and found that the technique has no radiation effect on the growth and activities of such living organisms. Consequently, an excellent study was conducted by Bouckaert et al.^[121] to monitor the real-time and dynamic activities of microbes in the soil after 22 d of exposure to X-rays and this also confirmed this assertion. This among other findings, confirm the effectiveness of using CT for studying such soil morphological and dynamic process. Investigation into the 3D micro-organization of

the soil-root-microbe system allowed Feeney et al.^[122] to critically examine the soil particle close to and away from the presence of roots in control pots and successfully develop a new model capable of investigating soil-plant-microbe interactions. In their model, the soil system represents the adaptive system with three defined and continuously interactive nodes; structure, microbes and plants. It revealed that at microscopic levels, the habitat of soil microbes and plant roots tends to change in its porosity and spatial correlation causing an increase in biological activity in a more porous soil structure. Further research in these areas could explain the point-to-point microscopic reactions in the soil. CT and biological experimentation needs to focus on imaging the living structural bodies of the individual microbes and their morphological responses to changing conditions in the soil such as decomposition as opposed to simply presenting results of their path configurations.

More recent work by Helliwell et al.^[123] on the influence of microbial activities on the structure and configuration of the soil also further supported this assertion. The authors approved the use of CT as an important tool for quantifying the activities of micro-living organisms (apart from earthworms) in the soil and their influence in the more dynamic rhizosphere soil-root interface. Suggestions on ways of monitoring the nutrient and water uptake of roots by CT technology and other relevant methods could pave a way for more in-depth experimentation leading to better understanding of the step-by-step processes in this zone.

Other studies on the pH dynamics in the rhizosphere by Blossfeld and Gansert^[124] showed marginal diurnal variations of pH along the roots elongation root, in particular. The combined effects of roots and rhizosphere organisms in a small volume of soil create bio-availabilities which may be completely different from that of the bulk soil^[125]. Bacterial and fungal diversity increases soil quality by affecting soil agglomeration and increasing soil fertility. They are both important in nutrient cycling and in enhancing plant health through direct or indirect means. In addition, a healthy rhizosphere population can help plants deal with biotic and abiotic stresses such as pathogens, drought and soil

contamination^[110,126]. For understanding bioprocesses in soils, especially those between roots, mycorrhizal fungi and micro-organisms in the rhizosphere, knowledge of the spatial and temporal dynamics of the physical and chemical conditions of the root–rhizosphere–soil interface is essential^[124]. Lilje et al.^[107] also developed an in vitro 3D alternative approach for evaluating how fungi grow in the soil medium using CT systems.

4 Summary and concluding remarks

CT offers a great opportunity for 3D characterization of soil-roots. Advantages of using the technique include: 1) It is suitable for 3D non-destructive sequential way of measurement and monitoring processes within the soil media without any outside disturbance over time and samples can be grown or used for other purposes after the experiment; 2) Most recent scanners are relatively fast and accurate in obtaining high quality images; 3) Scanning flexibility of the same sample at multiple energy levels is possible^[35].

From this literature review, it is proven that amidst challenges, researchers have utilized CT as an effective tool for visualizing and quantifying soil-root processes. The quality of 3D root architecture CT images has improved over the past decade. The results show that the use of CT data is now easier to manipulate with the introduction of effective segmentation tools such as RooTrak, VGStudio Max and ImageJ softwares. Utilization of such tools and modeling procedures could be useful in providing more detailed soil-root and root-root sensitivities and response reactions. Additionally, studies on soil structure dynamics also indicated that minimal increase in compaction of soil in the rhizosphere, for instance, tends to enhance crop productivity. Further work on the use of different soil types and moisture content conditions effect on crop growth using the techniques could produce interesting findings.

In relation to the monitoring of water flow and the activities of other living organisms in the soil, CT has made significant contributions towards the characterization of such soil system features. Due to improvement in resolution and other technical parameters

of CT systems, changes in soil structure caused by moisture patterns, living and non-living organisms as well as chemicals in the rhizosphere and bulk soils can be monitored. The performance of soil microbes, earthworms and insects is a matter of current active interest among researchers. This paves the way for a more exciting exploration of the root geometry, morphology and processes in the soils^[127]. Hence, with the improvement in the configurations to micro- and nano-levels, more minute point-to-point features and activities of such organisms could be closely monitored.

Based on this review of works related to soil-root processes using CT, the following needs have been drawn for the future:

- Assessment of the mechanism of root water and nutrient uptake in relation to morphology and rhizosphere biophysics and chemistry.
- Development of models and algorithms to further evaluate the impact of water and salt stress on the structure and configuration of the soil and root growth.
- Further evaluation of the impact of high and low X-ray energies on roots, soil organisms and mineral matter.
- The continued improvement in resolution and reduction of beam hardening effect in CT systems.

With collaborative efforts, studies related to these in-situ root-soil interactions could be effectively performed. The current fast advancement in CT technology could lead to the full understanding of the dynamic processes within the soil.

Acknowledgements

We acknowledge that this work was supported by grants from the National Natural Science Foundation of China (No. 51475216), the National Science & Technology Pillar Program during the Twelfth Five-Year Plan Period (No. 2013BAD08B03), the Jiangsu Province Science and Technology Support Program of China (No. BE2012381 & No. BE2014373), the Priority Academic Program Development of Jiangsu Higher Education Institutions (SFE (2014) 37), the Jiangsu Province Synergistic Innovation Center Program of Modern Agricultural Equipment and Technology (No.

NZXT02201402), and the Fund for Independent Innovation of the Jiangsu Province Agricultural Science and Technology (No. CX(15)1033-5).

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