Subsoiling and crop rotation improve root growth of *Bt*-cotton in Vertisols

D. Blaise*, G. Majumdar, A. Manikandan, S. Santosh and K. Velmourougane

Department of Crop Production, ICAR-Central Institute for Cotton Research, Nagpur 440 010, India

Soil compaction is a major physical constraint in cotton production. At present, no information is available on the effects of compaction on the root growth and root anatomy of cotton (*Gossypium hirsutum* L.). Therefore, we studied the effects of subsoiling (shallow (SSS) and deep (DSS)) and crop rotation (pigeon pea (*Cajanus cajan*) – cotton (PCR) and radish (*Raphanus sativus*) – cotton (RCR)) on the root growth of cotton in deep Vertisols during 2017–19. Subsoiling significantly increased the shoot and root length. The root-to-shoot ratio was maximum in DSS (33%), followed by PCR (29%) at the vegetative stage. Scanning electron microscopy analysis of the roots indicated a large number of pores and less contraction of xylem and phloem in the subsoiled and rotation treatments than in the control. Furthermore, the SEM-EDAX spectra indicated a greater abundance of major, secondary and micronutrients in subsoiling and crop rotations compared to the control treatment.

Keywords: *Bt*-cotton, crop rotations, root growth, soil compaction, subsoiling.

SOIL compaction is a process of compression of soil particles into smaller fractions. This reduces pore spaces with a concomitant increase in bulk density coupled with a decline in soil hydraulic conductivity affecting air and water movement in the soils[1], deteriorating soil health and consequently decreasing crop yield[2]. Though anthropogenic activities, including mechanization (conventional and mechanical tillage operations), are known to be a primary cause of soil compaction[3], the natural composition of soils (clay, sand, sand and organic matter content) and the soil-forming process also play a major role in soil compaction[4]. It is well documented that compacted soils affect the porosity, water infiltration, soil biology and crop emergence as well as root growth resulting in poor crop performance[5,6].

Though India accounts for approximately one-fourth (13.3 million hectares) of the world's cotton area, average productivity is low (484 kg lint ha^{-1}) compared to the world average (765 kg lint ha^{-1}). One of the main factors responsible for low yields is poor soil fertility and soil physical constraints[7]. In India, cotton is more susceptible to soil compaction, as it is typically grown in Vertisols, which have more than 50% clay content. Moist soil conditions coupled with the use of heavy machinery for land preparation in cotton-growing regions further accelerate soil compaction through physical pressure on the subsoil, resulting in poor crop performance[8]. Several agronomic interventions such as subsoiling, crop rotation and intercrop have been used to combat soil compaction[9]. Subsoiling in cotton reduced the negative effects of soil compaction and improved soil properties and cotton yield[10]. However, the energy requirements and costs involved with subsoiling are a concern, especially for deep soils[11]. Therefore, alternative methods such as growing cover crops along with conservation tillage were considered and reduced soil compaction effects[12,13]. Although several reports on the ill-effects of soil compaction on cotton productivity are available[14], few have reported adverse effects on the root growth of cotton crops. We hypothesized that subsoiling and crop rotation could positively affect the root growth of cotton in compacted soil. Therefore, we studied the effects of subsoiling and crop rotation on the root anatomy of cotton using scanning electron microscopy (SEM), energy dispersive X-ray analysis (EDAX) and Raman spectroscopy in a field experiment on rainfed Vertisols.

Materials and methods

Site description, experimental layout and treatments

Field experiments were conducted during 2017–18 to 2018–19 at ICAR-Central Institute for Cotton Research (CICR), Panjari Farm, Nagpur, Maharashtra, India (21°04′71″N, 79°04′40″E). This farm is situated at 309 m amsl and has a mean annual rainfall of 1026 mm. The experimental site had medium deep black soil (Typic Haplustert) classified as sub-humid moist bioclimate (10.2) under the agro-ecological sub-regions of India. The topsoil (0–30 cm) had 72% clay texture with 4.5% free calcium carbonate content. The soil was moderately alkaline in reaction (pH 7.8) and non-saline (electrical conductivity 0.27 dS m^{-1}) with a bulk density of 1.52 g cm^{-3}. The soil had low organic carbon (4.0 g kg^{-1}), low available nitrogen (126 kg ha^{-1}), medium available phosphorus (15 kg ha^{-1}) and high exchangeable potassium (744 kg ha^{-1}). The secondary and micronutrient status showed medium available sulphur (14.6 mg kg^{-1}), high available magnesium (350 mg kg^{-1}), medium available

*For correspondence. (e-mail: blaise_123@rediffmail.com)

boron (1.75 mg kg^{-1}), high available copper (3.50 mg kg^{-1}), high available manganese (15.3 mg kg^{-1}), medium available zinc (0.62 mg kg^{-1}) and high available iron (7.52 mg kg^{-1}).

The *Bt*-cotton hybrid (*Gossypium hirsutum* L, Ajit 155 BGII) was manually dibbled with the onset of monsoon in June, with a spacing of 90 × 60 cm using a line marker. The field experiment was laid out in a randomized complete block design with five treatments (T1: shallow subsoiling (SSS), T2: deep subsoiling (DSS), T3: pigeon pea–cotton rotation, T4: radish–cotton rotation and T5: control (no subsoiling or crop rotation)). Every treatment had three replicates, with each treatment plot measuring 98 m^2. Subsoiling was done every year in T1 and T2 treatments. In DSS and SSS, a subsoiler was run in the plots one month before sowing during both the years of study, to a depth of 0.40–0.45 and 0.25–0.30 m respectively. Soil compaction-breaking crops such as pigeon pea and radish were sown in rotation with cotton. In 2017–18, six plots were sown with cotton, three with radish and three with pigeon pea. In the following year (2018–19), radish and pigeon pea were sown in cotton plots of the previous year, and cotton was sown in three plots, each of radish and pigeon pea. In all treatments, recommended agronomic practices were followed for the crops with report to inter-cultural, nutrient and pest management[15].

Root anatomy and elemental composition analysis using SEM and EDAX

For SEM analysis, the cotton plants from different treatments were uprooted at the boll development stage and the root samples were cleaned with double-distilled water before processing. A thin root section (<0.1 mm) was taken, leaving the top 25 cm of the taproot, followed by dehydration in an oven for 30 min (45°C). For imaging, the root sections were mounted on the surface of an aluminium stub, sputter-coated with gold under argon gas and visualized at 1 mm, 300, 100 and 50 μm using SEM (FEI Quanta 250). The elemental composition of the root samples from different treatments was analysed using EDAX microanalysis at 100–200 μm.

Soil functional group analysis through Raman spectroscopy

The rhizosphere soil samples (0.5 mm) from different treatments were collected and oven-dried for 30 min (60°C) to remove excess moisture. To avoid artifacts of fluorescence, the experimental soils were analysed under dark conditions for the functional groups non-destructively using Raman spectroscopy (Raman Spectroscope Model R-3000 QE TM). Briefly, the powder-dried soils were kept in a polybag vial, and the Raman shifts (spectral range 200–2000 cm^{-1}) were recorded for qualitative and quantitative information at an interval of 1 cm^{-1}. After recording data under dark conditions, soil Raman spectra were collected in three replications to reduce the influence of red-light fluorescence for the elimination of spike. The laser wavelength was 785 nm produced by a solid-state diode laser operated at 100 mW at the source, which measures the spectral bands within the integration time of 2-sec exposure. Background, baseline and noise correction are pre-processing steps that were done to remove interfering signals from the samples. Background correction was done by polyfit smoothing to remove high-frequency noise. Derivatives were used to correct and remove unimportant baseline signals. After raw data retrieval, the refined graphs were prepared using Origin 6.0 version. Chemometric and data interpretation analysis of spectral shifts and stripping was done by a combined approach of empirical and tabular data[16,17].

Statistical analysis

Experimental data in triplicate related to the effects of subsoiling and crop rotation on the root attributes of cotton were statistically analysed by one-way analysis of variance (ANOVA) using WASP version 2.0 (Web Agri Stat Package, Indian Council of Agricultural Research, New Delhi). Significant differences in treatment means were differentiated by Tukey's honestly significant difference test at $P \leq 0.05$.

Results and discussion

Effect of subsoiling and crop rotation on shoot and root attributes of cotton

DSS and pigeon pea rotation significantly enhanced the shoot and root attributes of *Bt*-cotton than the control at the vegetative and squaring stages ($P < 0.05$) (Table 1). DSS and pigeon pea rotation enhanced shoot length by 26% and 22% respectively, compared to the control at the vegetative stage. While at the squaring stage, DSS and pigeon pea rotation enhanced shoot length by 47% and 28% respectively. A similar trend was observed with regard to root depth. DSS and pigeon pea rotation plots had deeper roots (DSS: 67% and 57% and pigeon pea rotation: 50% and 41% respectively, for vegetative and squaring stages) than the control. Higher root : shoot ratio was observed in DSS (33%), followed by pigeon pea rotation (29%) at the vegetative stage. However, PCR followed by RCR showed a higher root : shoot ratio at the squaring stage (Table 1).

Soil compaction is one of the primary factors responsible for deterioration of soil health and subsequently crop yield. Intensive use of heavy machinery along with cotton monocropping are probable reasons for enhanced soil penetration resistance and soil compaction problems. Further, intensive and incorrect use of machinery causes rooting problems like poor root growth and decreased cotton yield[18]. We observed cotton roots in the subsoiled and crop

Table 1. Subsoiling and crop rotation on shoot and root attributes

	Shoot length (cm)		Root length (cm)		Root : shoot ratio	
Treatment	Vegetative	Squaring	Vegetative	Squaring	Vegetative	Squaring
SSS	30[b]	35[c]	61[d]	80[c]	2.03[d]	2.29[b]
DSS	34[a]	44[a]	90[a]	96[a]	2.65[a]	2.18[c]
PCR	33[a]	38[b]	85[b]	90[b]	2.58[b]	2.37[a]
RCR	29[c]	33[d]	70[c]	76[d]	2.41[c]	2.30[b]
Control	27[d]	30[e]	54[e]	64[e]	2.00[d]	2.13[d]
Standard error mean	1.28	2.38	6.86	5.57	0.13	0.04
Standard deviation	2.88	5.33	15.3	12.4	0.3	0.09

SSS, Shallow subsoiling; DSS, Deep subsoiling; PCR, Pigeon pea–cotton rotation; RCR, Radish–cotton rotation; Control, No subsoiling or rotation. All values are mean of three replications. Means followed by a common letter in a column are not significantly different according to Tukey's honestly significant difference test ($P < 0.05$).

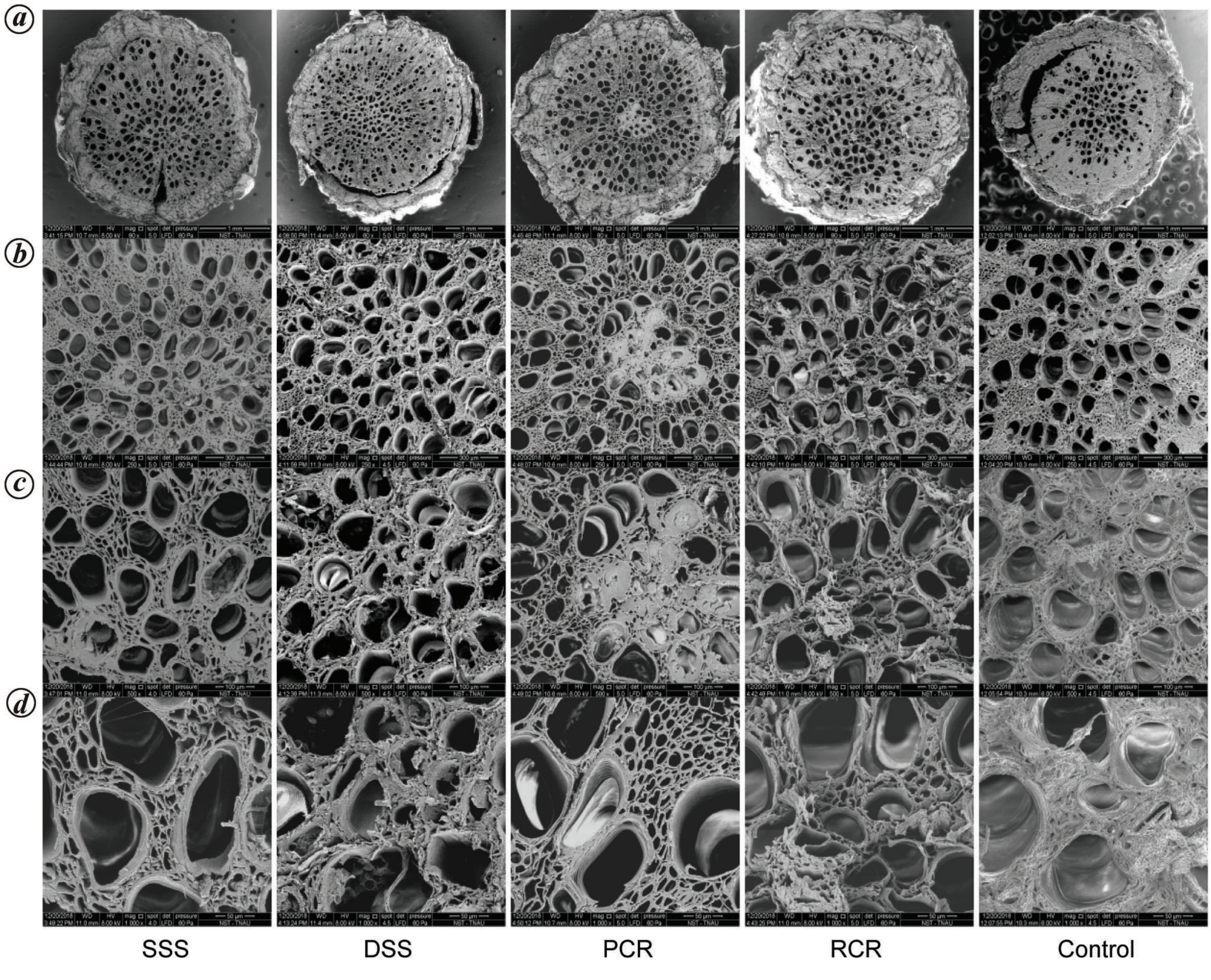
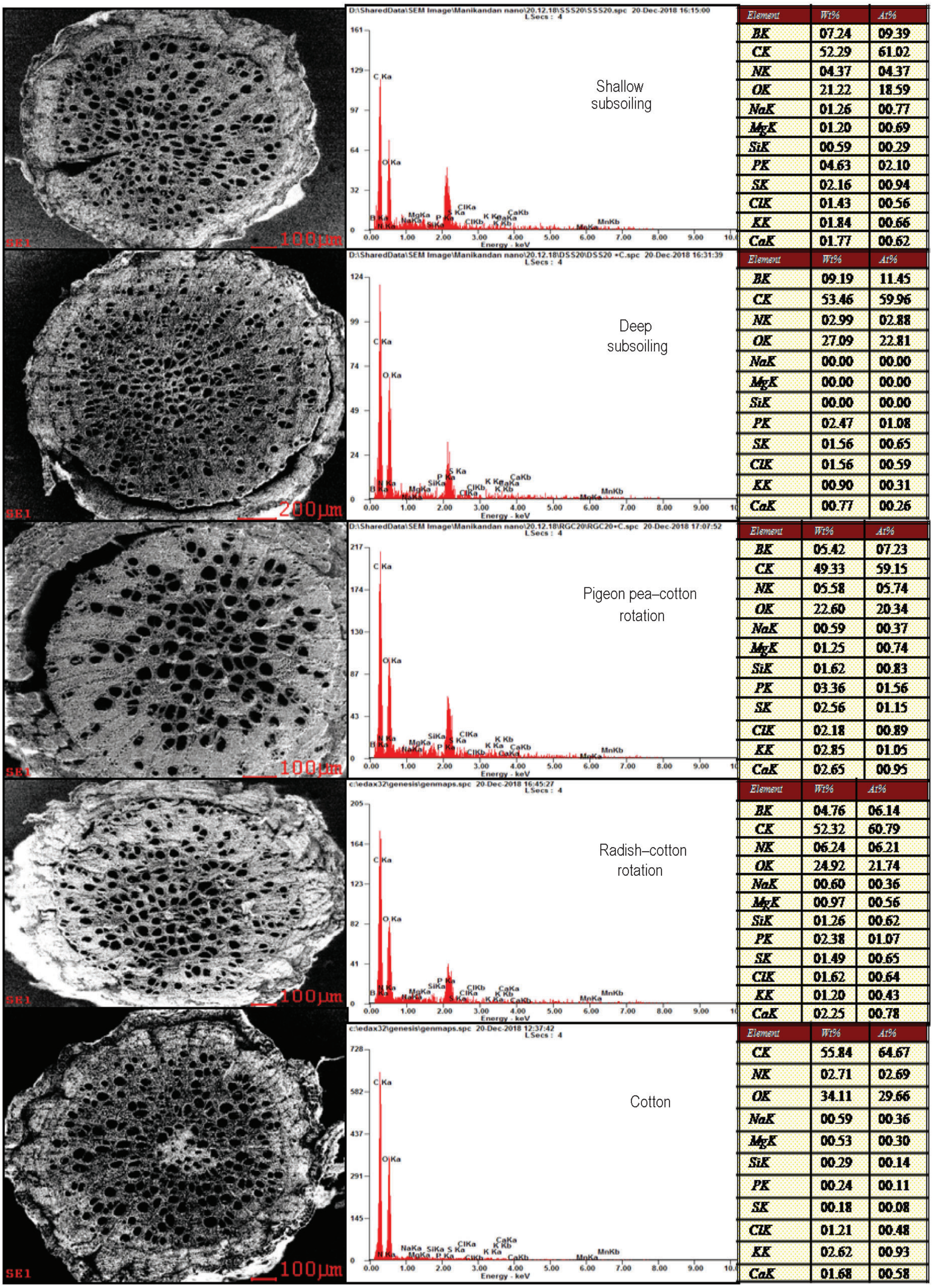


Figure 1. Scanning electron photomicrographs depicting the effects of subsoiling and crop rotation on cotton root anatomy. SSS, Shallow subsoiling; DSS, Deep subsoiling; PCR, Pigeon pea–cotton rotation; RCR, Radish–cotton rotation; Control, No subsoiling or rotation. Magnification: (***a***) 1 mm, (***b***) 300 μm, (***c***) 100 μm, (***d***) 50 μm.

rotation treatments to grow deeper than the control, which is supported by earlier findings on compaction effects in cotton[10,12,19]. Subsoiling (50–55 cm) followed by mouldboard plough decreased penetration resistance and increased cotton yield[20]. Similarly, subsoiling tillage up to 35 cm enhanced soil physical properties and increased maize yields in the North China Plain[21]. In winter wheat production, subsoiling practice decreased soil bulk density and



Shallow subsoiling

Element	Wt%	At%
BK	07.24	09.39
CK	52.29	61.02
NK	04.37	04.37
OK	21.22	18.59
NaK	01.26	00.77
MgK	01.20	00.69
SiK	00.59	00.29
PK	04.63	02.10
SK	02.16	00.94
ClK	01.43	00.56
KK	01.84	00.66
CaK	01.77	00.62

Deep subsoiling

Element	Wt%	At%
BK	09.19	11.45
CK	53.46	59.96
NK	02.99	02.88
OK	27.09	22.81
NaK	00.00	00.00
MgK	00.00	00.00
SiK	00.00	00.00
PK	02.47	01.08
SK	01.56	00.65
ClK	01.56	00.59
KK	00.90	00.31
CaK	00.77	00.26

Pigeon pea–cotton rotation

Element	Wt%	At%
BK	05.42	07.23
CK	49.33	59.15
NK	05.58	05.74
OK	22.60	20.34
NaK	00.59	00.37
MgK	01.25	00.74
SiK	01.62	00.83
PK	03.36	01.56
SK	02.56	01.15
ClK	02.18	00.89
KK	02.85	01.05
CaK	02.65	00.95

Radish–cotton rotation

Element	Wt%	At%
BK	04.76	06.14
CK	52.32	60.79
NK	06.24	06.21
OK	24.92	21.74
NaK	00.60	00.36
MgK	00.97	00.56
SiK	01.26	00.62
PK	02.38	01.07
SK	01.49	00.65
ClK	01.62	00.64
KK	01.20	00.43
CaK	02.25	00.78

Cotton

Element	Wt%	At%
CK	55.84	64.67
NK	02.71	02.69
OK	34.11	29.66
NaK	00.59	00.36
MgK	00.53	00.30
SiK	00.29	00.14
PK	00.24	00.11
SK	00.18	00.08
ClK	01.21	00.48
KK	02.62	00.93
CaK	01.68	00.58

Figure 2. EDAX spectra showing relative abundance of elements in cotton roots in the subsoiling, crop rotation and control treatments.

Table 2. Subsoiling and crop rotation on shifts in Raman spectra

Raman spectra (cm^{-1}) band assignments and their intensity										
SSS		DSS		PCR		RCR		Control		Functional groups
619	s	–		619	w	626	w	619	m	υ (C–S) aliphatic (s)
655	m	658	w	655	w	658	m	655	w	υ (CC) alicyclic, aliphatic chain vibrations (m), SiO_4 stretching
–		–		–		685	m	–		C–H out-of-plane vibrations
759	m	761	m	759	m	761	m	761	s	C–H out-of-plane vibrations
823	s	821	m	–		821	m	819	s	C–H out-of-plane vibrations
899	m	–		–		899	m	899	w	ν (C–C) vibrations
912	w	916	w	–		912	w	912	w	C–H out-of-plane vibrations
981	w	981	w	981	w	981	w	981	w	Aromatic C–H out-of-plane deformation
1029	w	1031	w	–		1031	m	1020	m	C–C stretch,
1070	m	1072	w	1074	W	1072	m	1072	m	C–O stretch,
1084	s	–		–		–		1084	m	C–O–H deformation
1178	w	–		–		1178	w	–		C–C stretch, C–X
1195	w	–		–		–		–		Amorphous C vibrations
1257	s	1250	w	–		–		1250	w	CH_2 deformations
1357	s	1331	w	1331	s	1331	w	1357	s	Symmetric CO stretch Diamond C=C (D band)
1378	m	–		–		1378	s	1378	s	C–CH_3 deformations
1418	w	–		–		–		–		Amorphous C vibrations
1444	m	1446	m	1446	s	1446	w	1446	m	Inorganic carbon (carbonates)
1465	s	1465	m	1465	s	1465	m	1465	s	C–H bending, C–H_2 def
1519	m	1519	m	1519	s	1519	s	1519	s	Amorphous C vibrations
1537	w	–		–		1537	w	1537	w	Graphitic C=C (G band)
1568	s	1567	m	1567	s	1567	s	1567	m	Aromatic ring chain vibration, C=C, amide II, N–H, C–N
1617	s	1615	s	1615	m	1617	s	1617	s	Aromatic ring stretch C=C
1672	w	1672	s	1672	m	1673	m	1672	m	Silicon, amide, C=O, C–N, N–H, C=C
–		1697	w	–		–		1697	w	Carbonyl stretch C=O
1716	m	1716	s	1716	m	1716	m	1716	m	C=O vibrations
–		1754	m	1756	m	1756	m	1754	m	C=O vibrations
1805	m	1805	m	–		1805	m	1805	s	C=O stretching vibrations
1861	m	1859	m	1857	s	1857	s	1857	s	C=O stretch
–		–		–		–		1903	m	C=X, C≡X
1909	s	1909	m	1909	s	1909	s	1909	s	
–		–		–		–		1940	w	
1952	s	–		–		–		1952	m	

s, Strong; m, Medium; w, Weak.

decreased soil penetration resistance, resulting in improved root morphology, enhanced root enzyme and hormonal activities, delayed root senescence and increased yields[22].

Root anatomy and elemental composition

SEM images of root samples from different treatments indicated a significant change in root attributes, including pore numbers, shape, size and orientation. Subsoiling (SSS and DSS) and crop rotation (pigeon pea and radish) treatments showed a greater number of pores compared to the control (Figure 1). While subsoiling and crop rotation treatments had a minimum contraction of xylem and phloem, pore contraction was maximum in the control treatment. SEM-EDAX spectra revealed a higher number of elements in SSS, pigeon pea and radish (12), followed by the control (11) and DSS (9). Major, secondary and micronutrients were in greater abundance in subsoiling and crop rotation treatments than in control. Interestingly, sodium, magnesium and silicon were absent in the EDAX spectra of DSS, while the control treatment showed the absence of boron (Figure 2). In the elemental composition, crop rotation treatments had higher nitrogen (N) content than the subsoiling treatment. Among crop rotations, radish and pigeon pea had N content of 6.24% and 5.58% respectively, than the control (2.7%). With regard to phosphorus (P), SSS, DSS, pigeon pea and radish had 19-, 10-, 14- and 10-fold higher P compared to the control. Subsoiling and radish treatments showed lesser potassium (K) content compared to the control, in which the K content was reduced by 42%, 191% and 118% respectively, for SSS, DSS and radish. Pigeon pea rotation increased K content by 9% over the control. Facilitation of deep crop root growth in the rotation and subsoiled treatments probably contributed to K extraction from the clay interlayers, unlike cereal crops like wheat

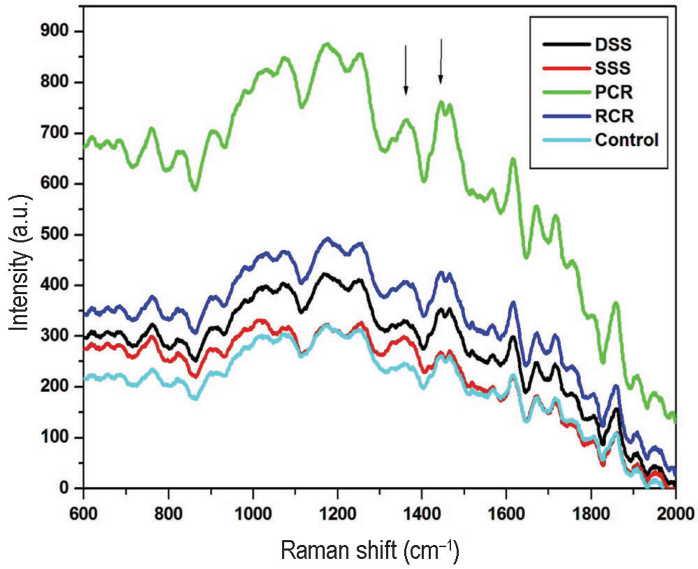


Figure 3. Effects of subsoiling and crop rotation on functional groups of the rhizosphere soil represented through shifts in Raman spectra.

and rice[23,24]. However, this was not the case with pigeon pea rotation. A probable reason could be the substantial leaf litterfall in the pigeon pea plots that may have contributed to K recycling[25]. Subsoiling (SSS and DSS) and crop rotation treatments showed higher sulphur (S) accumulation in the root tissues, ranging from 8- to 14-fold. Soil compaction reduces pore size[26] and modifies rhizosphere soil chemistry, water and nutrient mobilization[27–29]. Furthermore, in a compacted soil, nutrient availability is affected due to reduced oxygen[30].

Raman spectroscopy and soil functional groups

Raman spectra of the rhizosphere soils revealed that the Raman intensity was maximum for crop rotation (PCR (2.07 times) and RCR (0.66 times)) compared to subsoiling (DSS (0.38 times) and SSS (0.10 times)) and the control, confirming higher C–C, C–O, C–N, C–O–C spectral vibrations in subsoiling and crop rotation treatments. Higher aliphatic (C–S) group vibrations (626 cm^{-1}) were recorded in radish, while higher alicyclic (C–C) chain vibrations (658 cm^{-1}) were recorded in radish and DSS treatments. Medium C–H out-of-plane vibrations (685 cm^{-1}) were observed only in the radish rotation plots (Table 2 and Figure 3). Raman spectroscopy and Fourier transfer infrared spectroscopy are frequently used to study the effects of land-use change on the mineral composition of soils, including soil organic carbon status[31–33].

Conclusion

At the end of two years of subsoiling (shallow and deep) or crop rotation (pigeon pea–cotton and radish–cotton), the root attributes of cotton, such as shoot/root growth and root : shoot ratio were greater, compared to the control (no subsoiling or crop rotation) in deep Vertisols. Subsoiling and crop rotation also enhanced the pores, reduced the contraction of xylem and phloem, and improved the accumulation of major, secondary and micronutrients in the roots than the control. Thus, subsoiling and crop rotation can be explored as an ecofriendly and alternative technology to combat soil compaction in Vertisols.

Conflict of interest: The authors declare that there is no conflict of interest.

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ACKNOWLEDGEMENT. We thank the Indian Council of Agricultural Research, New Delhi and the Director, ICAR-CICR, Nagpur for financial assistance to carry out this study.

Received 21 January 2022; revised accepted 10 August 2022

doi: 10.18520/cs/v123/i7/874-880