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MINERALOGY OF RHIZOSPHERIC AND NON-RHIZOSPHERIC SOILS IN CORN FIELDS*

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Abstract—Technical limitations have restricted investigations of rhizosphere mineralogy. Various analytical techniques were applied to assess root-mineral associations and dynamics in natural soils under corn production. Soil samples were collected between four and five weeks after planting and included rhizospheric and non-rhizospheric soils, and undisturbed block samples containing corn root systems. Analytical techniques were applied and included; X-ray diffraction, optical microscope, SEM, EDXRA with SEM, transmission electron microscope (TEM), electron energy loss spectra with TEM, high-resolution transmission electron microscope (HRTEM) and microanalysis with HRTEM. The mineralogy of the rhizosphere differed from that of the bulk soil. Within the rhizosphere, minute platy particles which were mostly vermiculitic minerals, were particularly concentrated near or on root surfaces. These platy mineral particles were not attached to the entire area, but only to certain areas of root surfaces. Therefore, we report quantitative evidence for mineralogical changes in the rhizosphere in soil environments.

Key Words—Corn, Mineralogy, Non-rhizosphere, Rhizosphere.

INTRODUCTION

The rhizosphere is that zone in the soil where living plant roots interact with surrounding mineral, organic, and microbial components of the soil (Curl and Truelove 1986). Plant processes associated with root growth through soil, such as root exudation, production of mucilaginous materials, and the uptake of elements cause specific physico-chemical environments around roots that are quite different from those in the bulk of soil (Keller and Frederickson 1952). These interactions play an important role in determining plant nutrition and growth (Robert and Berthelin 1986). In addition, soil weathering processes are strongly influenced by the presence of roots and by their effects on rhizospheric microbial activity (Berthelin 1988). For example, vermiculitization of biotite by plant growth is known (Mortland *et al* 1956). Hinsinger and Jaillard (1993) and Hinsinger *et al* (1992, 1993) demonstrated that such a vermiculitization could occur within a matter of several weeks (even a few days in some cases) in the rhizosphere. Recently, April and Keller (1990a, 1990b) showed that the mineralogy of the rhizosphere differed from that of the bulk soil in forest soils. Few studies have addressed mineral-root associations and dynamics in natural soils. This is partly due to the lack of appropriate analytical techniques for the study of mineral associations on root surfaces. Our understanding of these associations and their role in the agrochemical cycles of agricultural systems is thereby limited.

A study was initiated to examine mineral-root associations and dynamics in natural soils. The purpose of this paper is to present (1) mineralogical comparison between the bulk and rhizosphere soils, (2) specific mineral association near and on root surfaces, and (3) plausible explanations and implications for the mineral association.

EXPERIMENTAL

Sampling sites and sample collection

Samples were collected from three sites near Ottawa, Canada (45°22′N, 75°43′W), and contained soils of various textures: loamy sand at Cobden (COB), clay loam at Pakenham (PAK), and sandy loam at Central Experimental Farm (CEF). COB and CEF sites had been in corn production for more than five years and PAK site for two years under conventional tillage practices (fall stalk chopping and mouldboard with spring cultivation). Samples were collected from plots established for corn hybrids performance testing (Dwyer *et al* 1991). Samples, nine replicates at each site, were collected between three and four weeks after planting when the corn was at the two-leaf stage and included various root-soil samples and undisturbed block samples containing corn roots. In the case of root-soil samples, each plant and its surrounding soil was excavated, sealed in a plastic bag, and stored in the dark at 2°C until processed. Kubiena boxes (Sheldrick 1984) were used to collect and maintain the block samples in an

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undisturbed condition. These boxes were made of galvanized iron and had openings at two ends with dimensions of 7.5 × 6.5 × 5 (depth) cm. A vertical soil profile was prepared near a detopped corn plant and Kubiena boxes were carefully inserted into the profile using hand pressure. The boxes were inserted so that the detopped corn plant was at the centre of the box. The surrounding soil was excavated and the rough soil surfaces at both ends of the box were trimmed. The open ends of the box were covered with precut pieces of plywood to secure the sample and then tightly sealed in a plastic bag to prevent moisture loss.

Sample preparations

Rhizosphere. In this study, rhizosphere samples consisted of a narrow core of soil, always less than 1 mm, associated with and adhering to corn root surfaces.

Transmission Electron Microscopy (TEM)

Fresh roots with adhering soil particles from root-soil samples were cut into several segments of 0.5 to 1.0 cm length within one day after sampling from fields. Samples for TEM were prepared by procedures similar to those described by Dawes (1971) and Hayat (1989). Briefly, the segments were immediately stabilized in 2% agar gel medium to maintain the spatial arrangements between rhizospheric components. The stabilized segments were fixed with 3% glutaraldehyde for 3 h in 0.1 M sodium cacodylate buffer at pH 7.2, post-fixed in 1% osmium tetroxide for 2 h in 0.05 M sodium cacodylate buffer, dehydrated in a series of graded ethanol, and embedded in Epon 812 and cured in an oven at 60°C for 36 hours. Ultrathin sections of 40–70 nm thickness were cut with a diamond knife on a Reichert Ultracut E ultramicrotome, collected on carbon coated grids and then stained with uranyl acetate and lead citrate before examination. It was extremely difficult to slice sandy rhizosphere samples properly, as many quartz grains tended to be shattered into conchoidal fragments during sectioning, causing the thin film to break up. Ultrathin sections of 30–40 nm were also prepared and collected on bare Gilder fine bar beehive style grids (G600HH), which provide high transmission value with maximum supporting strength for electron energy loss spectroscopy study.

X-Ray Diffraction (XRD)

Subsamples of rhizospheric and non-rhizospheric soils were obtained from the root-soil samples. Non-rhizospheric or bulk soil samples consisted of soil at least a few millimetres away from root surfaces and were obtained by briefly air-drying root-soil samples to allow separation of bulk soil from the soil adhering to roots. Rhizospheric soil samples were collected by removing adhering soil particles from air-dried root surfaces with a fine brush and consisted of particles from within 1 mm of the root surfaces. Relatively large mineral grains (>1 mm) were removed from the rhizospheric soil sample. Samples were further air-dried. Approximately 2 g aliquots were placed in a hardened steel vial to ground 45 μm with a Spex Mixer/Mill. These samples were also treated with 30% H_2O_2 on a water bath to remove organic matter (Sheldrick 1984). The weight loss due to this treatment was regarded as an approximate organic matter content. Due to relatively small amounts of organic matter, the difference in XRD patterns before and after treatment with H_2O_2 was small.

Scanning Electron Microscopy (SEM)

Undisturbed samples in Kubiena boxes were placed in an exchange chamber and sample water was gradually replaced with acetone for 8 weeks prior to impregnation with epoxy resin (Sheldrick 1984). Solidified impregnated blocks were sliced vertically into 4 to 5 mm thick slabs and observed by light microscopy. Areas suitable for further examinations were chosen and thin sections were prepared and examined without cover glass, under a polarized light microscope. Small portions of these thin sections were selected and prepared by a Buehler ISOMET low speed saw for further observations under an SEM. Each of the selected segments for thin sections was cut, mounted on an aluminum stub, coated with carbon and examined.

Sample analyses

X-ray Diffraction (XRD). Mineral composition of the ground rhizospheric and non-rhizospheric soil samples was determined quantitatively by comparing their characteristic XRD peak intensities with those of standard minerals. XRD analysis was accomplished by a random mount method and supplemented by an oriented sample method. Ground samples were packed in a 17 mm-diam cylindrical window with 12 mm depth drilled in a square Plexiglas plate. Ground samples were remounted in the sample holder for replicate determinations. Oriented specimens were prepared by drying a 1 ml liquid suspension containing 30 mg ground sample on a 25 mm × 30 mm glass slide. Glycerol solvation with 2% glycerol/water solution was used to detect expansible physillicates such as vermiculite and smectite. Air-dried oriented samples were heated at 550°C for 0.5 hours to distinguish chlorite from vermiculite. These variously pretreated and oriented sample and random mount techniques provided optimum conditions for identification and quantification of phyllosilicates. For some phases of this study, the clay fraction of a rhizospheric soil sample was separated and saturated with Mg^{2+}. The oriented specimens of the fraction were similarly prepared and pretreated as described above. All XRD patterns were recorded using a Scintag PAD V diffractometer with Co radiation and a graphite monochrometer. Amounts of minerals were estimated from diffraction peak intensities according

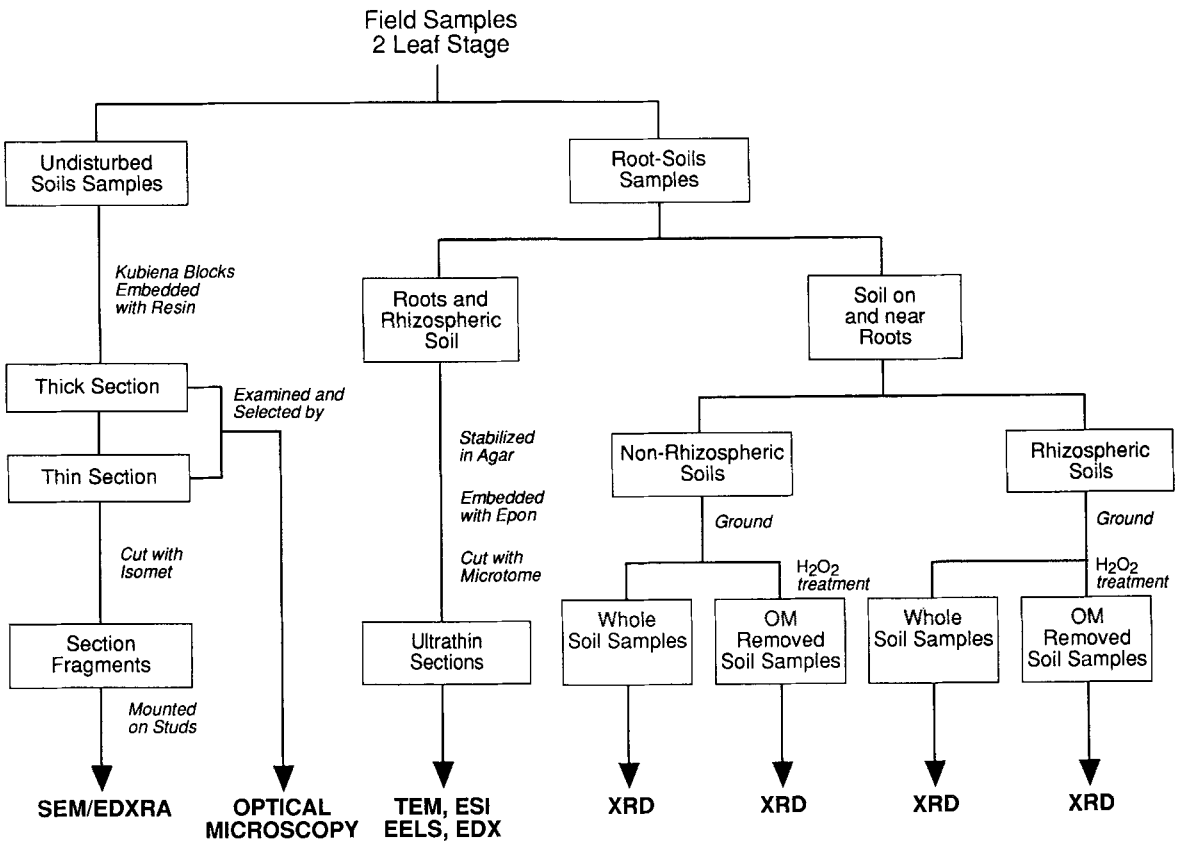

Figure 1. Scheme for sample preparations and subsequent analyses.

to procedures similar to those described by Kodama *et al* (1977).

Electron microscopy. Stereoscanning electron microscopic (SEM) observations with spectrochemical analysis were made on thin sections prepared from undisturbed soil samples by an Akashi ISI-DS-130 SEM equipped with a Tracor Northern energy-dispersive X-ray analyzer (EDXRA). TEM observations of root and adhering rhizospheric soil were made in Philips EM300 or Zeiss EM902A transmission electron microscopes. The latter was equipped with an image processing system (Kontron, Vidas) on-line with a high resolution image intensifier camera attached to the column. Stained sections were examined in the Zeiss EM902A to obtain electronspectroscopic images (ESI). Element distribution maps were obtained by an electron energy loss spectroscopic (EELS) method (Castaing and Henry 1962; Ottensmeyer and Andrew 1980). The spectra were obtained by subtracting digitized ESI of unstained ultrathin sections below the ionization edge from that taken above the same edge, with the background value of an epoxy section void of specimens taken into account (Heinrich *et al* 1990; Lehmann *et al* 1990; Busch *et al* 1993). Ionization edge of L-shell for respective elements was used in our study. The Hitachi H-8000 was operated at 200 KV to obtain lattice images of minute mineral grains attached to roots and to make elemental analysis of these grains with the aid of an energy-dispersive X-ray analyzer, which was attached to the electron microscope.

The sample collection, sample preparation including pretreatments, and specific analytical techniques described above are summarized in a flow chart (Figure 1). Statistical analyses were prepared using General Linear Models (GLM) procedures of SAS (SAS Institute, Cary, N.C., USA).

RESULTS AND DISCUSSION

Mineral assemblages found in non-rhizospheric (bulk) soils from the three sites were very similar. They consisted of quartz, microcline, plagioclase, amphibole, mica, vermiculite and interstratified clay minerals with or without trace amounts of chlorite and smectite. Amounts of these minerals were estimated from triplicate XRD data for respective sites. Quartz, microcline, plagioclase, amphibole and mica made up nearly 83–87% of the total soil component. The remaining minerals took only about 5%. Besides crystalline minerals, the non-rhizospheric soils contained 2% organic matter and about 1% noncrystalline inorganic com-

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Table 1. Mineral composition[1] of rhizospheric (R) and non-rhizospheric (NR) soils from Cobden (COB), Pakenham (PAK), Central Experimental Farm (CEF) to show main rhizosphere and site effects on them.

Site[2]	Sample	Quartz	Microcline	Plagioclase	Amphibole	Phyllosilicates			
						Mica	Vermiculite	Inter-strat. minerals	Total phyllosilicates
A) Main rhizosphere effects									
All	NR[3]	46.5 a[4]	4.9 a	9.5 a	13.6 a	9.2 a	2.8 a	2.0 b	14.0 b
All	R	36.4 b	4.1 a	12.6 a	13.2 a	9.2 a	4.6 a	6.3 a	20.1 a
B) Main site effects									
COB	all	43.8 a	4.3 a	11.3 ab	11.0 a	8.3 a	4.5 a	3.5 a	16.3 ab
PAK	all	40.5 a	4.4 a	14.2 a	15.0 a	8.8 a	1.7 b	3.7 a	14.2 b
CEF	all	40.2 a	4.8 a	7.7 b	14.3 a	10.5 a	4.8 a	5.3 a	20.7 a

[1] Weight %; organic matter (2% in NR, 3% in R) and noncrystalline inorganic soil component (~1% in both) are not included.
[2] Sites as described in text.
[3] NR = Non-rhizosphere, R = rhizosphere soil samples.
[4] Mean separation by Least Square Means difference within a section and column at $p < .05$ indicated by different letters; (A) n = 9, (B) n = 6.

pounds. Approximately 5–9% underestimations might have resulted from comparing peak intensities of well-crystalline standard minerals with those of respective minerals in soils.

In rhizospheric soils, similar results were obtained except that the content of non-phyllosilicates plus mica was about 5% lower whereas the content of vermiculite and interstratified clay minerals was about 6% higher than those in the non-rhizosphere. The rhizospheric soils also contained 3% organic matter and about 1% noncrystalline inorganic compounds. Therefore, in spite of three different sites, soil components were rather similar. However, there appeared to be consistent differences in the mineralogy between rhizospheric and non-rhizospheric soils. To test this, statistical analyses using GLM procedures were applied and some of the results are given in Table 1. The similarity of soil components in the three different sites reflected on mean separations indicating that amounts of plagioclase at CEF and vermiculite at PAK were only significant differences. This incidental situation may add more validity to the results of statistical analysis on main rhizosphere effects among the three sites. The analysis (Table 1) shows consistent differences in the mineralogy between rhizospheric and non-rhizospheric soils. In the rhizosphere, quartz was less concentrated while interstratified clay minerals were more concentrated. As the interstratified clay minerals were chiefly composed of mica and vermiculite, the content of vermiculite and vermiculitic mineral components altogether in the rhizosphere was higher than in the non-rhizosphere.

Scanning electron micrographs of six thin sections provided some morphological and textural details of mineral grains near and within the rhizosphere. Figure 2 gives examples of such observations. As seen in Figure 2-1, the distribution of mineral grains was generally more dense near the roots, whereas there were more voids as the distance from the roots increased. The morphology of mineral grains and associated EDXRA data (Figures 2-4 to Figure 2-8) allow identification for most mineral grains such as quartz, amphibole, plagioclase and microcline (Figure 2-9). Due to the similar chemical composition, microcline might be confused with muscovite. In the present case, however, the morphology supported the identification of microcline for the grain in Figure 2. The observed area for the distribution of identified mineral grains (Figure 2-9) was approximately 0.06 mm^2 on the surface of the root. Although the XRD data indicated the concentration of phyllosilicates in the rhizosphere, SEM-EDXRA examinations on this and other thin sections failed to positively identify any phyllosilicates, but were useful to view modes of mineral association within the rhizosphere. It was noteworthy that small silica precipitates were observed within the roots.

Transmission electron microscopic (TEM) observations on mineral particles present very near and on root tissues indicated the presence of clay particles. The clay particles were often observed as aggregates trapped in pockets or depressions that were formed, or outlined by the external tissues of roots and root hairs (Figure 3A). In some cases, multiple layers of flaky particles (Figure 3B) or individual flaky and elongated particles (Figure 3C) were attached on a stretch of root surface. It was interesting to observe that these associations of clay particles with roots were always concentrated on limited areas of root surfaces only. Since the mucilages exudated from roots are considered to be effective soil binding agents (e.g., Foster 1981; Wullstein and Pratt 1981), these limited areas of root surfaces with adhering clay particles were suspected to be those where the mucilage had accumulated. In some instances, TEM showed that clay-size particles penetrated into the mucilage layer of root (Figure 3D).

Although the morphology of flaky particles indicated

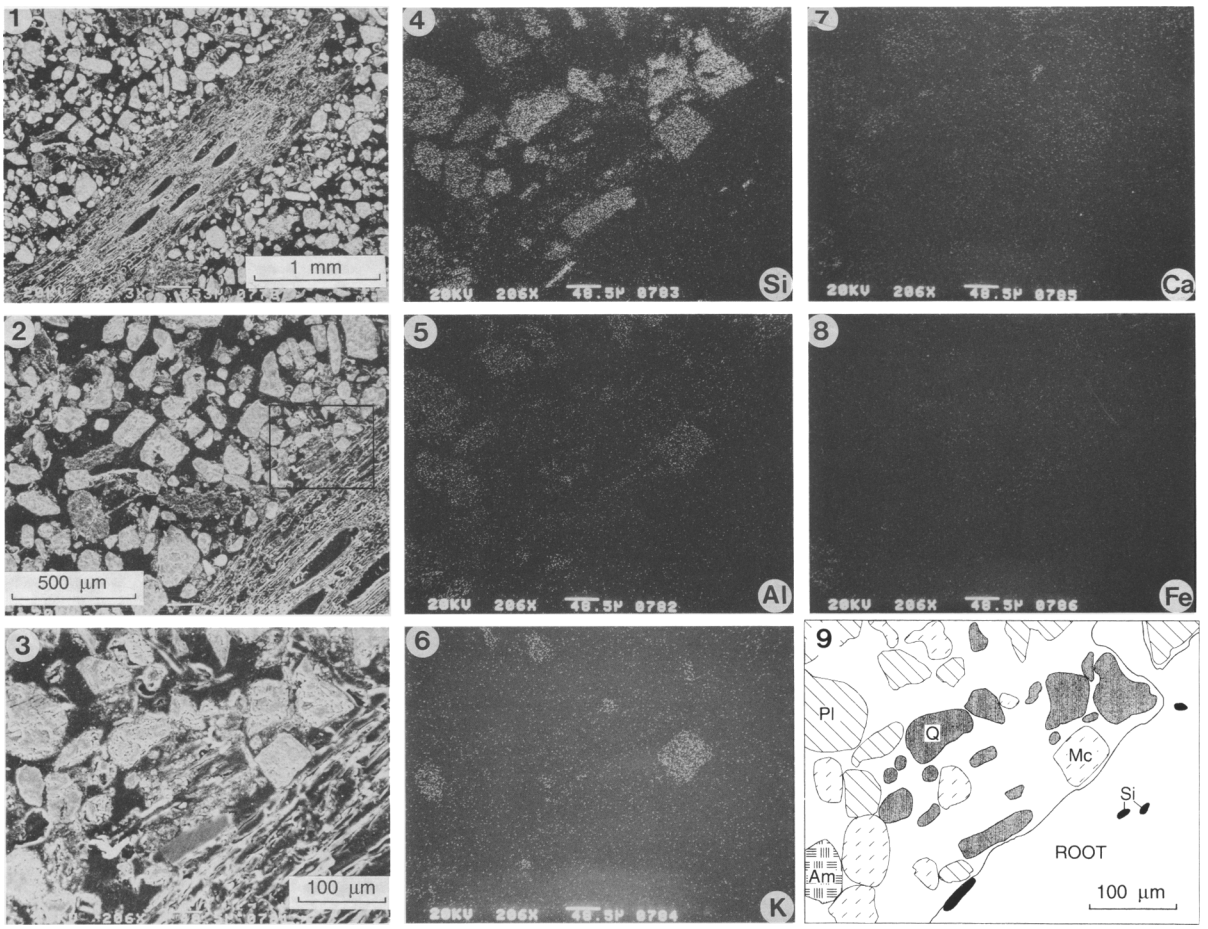

Figure 2. Scanning electron micrographs (1, 2 and 3), energy-dispersive X-ray analyses (4 to 8) of an enlarged section shown in 3, and sketch (9) showing identified mineral grains. Abbreviations used in the sketch are: Q; quartz, Mc; microcline, Pl; plagioclase, Am; amphibole, Si; silica precipitate most likely opaline silica.

that they appeared to be tiny particles of certain phyllosilicates, the morphology alone could not confirm that this was the case. The TEM data supplemented by EDX provided us with a better mineral speciation for the particles near and on root tissues. An example is given in Figure 4. Silicon and Al were the major elements of all particles examined. Particle 3 is probably plagioclase as Na and Ca are associated with the major elements. In particle 7, on the other hand, Mg, K and Fe are present, suggesting that the particle may be biotite. High Al and Fe content with particle 8 indicated that an aluminosilicate particle is possibly coated or closely associated with Al and Fe oxides or oxyhydroxides. Particle 9 consists of Si and Al only. Particles 4, 5 and 13 contain small amounts of Mg, K and Fe. Particles 11 and 12 do not have K but Fe, whereas particle 14 does not contain Fe but a small amount of K. These findings were a little surprising, because mica particles were more commonly anticipated from XRD data. The K content of the particles concerned was considerably low if they were mica. Particles 4, 5 and 13 were considered to be K-depleted biotite in an advanced stage of weathering, whereas particle 14 resulted a K-depleted illite. This, however, appeared to be a special case in this observed area, because other particles contained more or less Fe and Mg, except for particle 12 in which no Mg was present. Therefore, particles 11 and 12 were interpreted as a dioctahedral vermiculite closely associated with free iron oxides. Although the mineral composition given in Table 1 does not specify the mica, further XRD data of a clay fraction separated from a rhizosphere soil showed very weak second-order basal reflection at 0.5 nm, as compared with the first-order basal reflection at 1.0 nm, suggesting that the majority of mica may be biotite or its mixture with a small amount of illite.

According to the XDR data (Table 1), the rhizosphere contained approximately twice as much mica as vermiculite. Interstratified clay minerals were mostly of a mica/vermiculite type. Considering respective component layers from the interstratified minerals, mica

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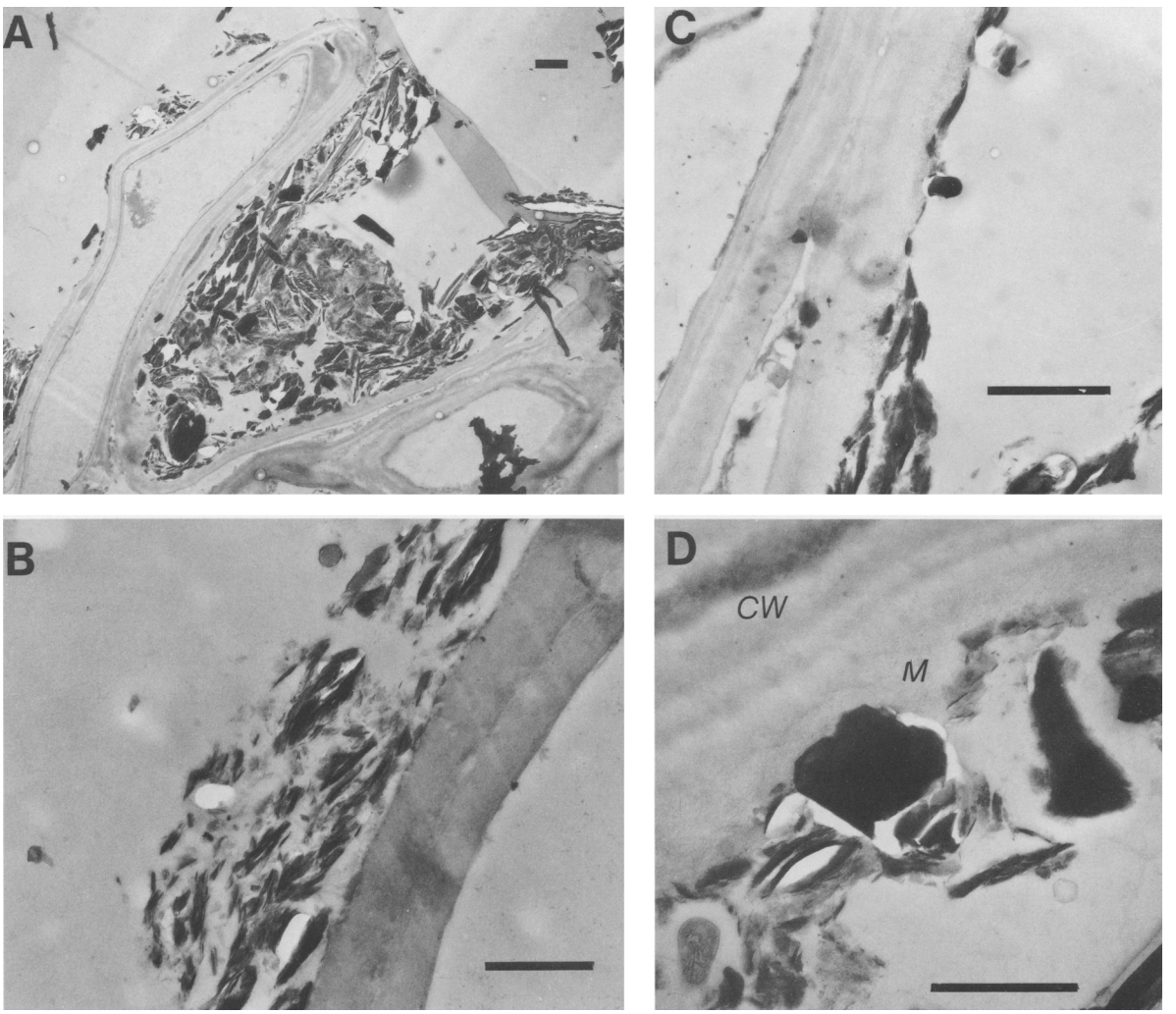

Figure 3. Transmission electron micrographs. CW = cell wall, M = mucilage. Scale indicates 1 μ.

may still exceed vermiculite. Even particle 13, which showed one of the highest peaks for K among 10 particles shown in Figure 4 appeared to be K-depleted mica or vermiculitized mica. This indicated that the mineral distribution very near or on root tissues was quite different from that in the other parts of the rhizosphere.

The TEM-EELS micrographs (Figure 5) gave further support that flaky particles attached on root surfaces were mostly aluminian vermiculite minerals. The first example at the top (Figure 5) shows that tiny particles (black) attached to root tissues consist only of Si and Al (white areas on respective element mapping). Similar results were also obtained with the second example at the bottom of Figure 5.

In a few cases, we managed to obtain lattice images of flaky particles attached to root tissues by TEM. Most lattice images were not well-defined and irregular (Figure 6). The observed periodicity of the lattice image ranged from 1.0 to 1.4 nm which was in accordance with anticipated periodicity of collapsed vermiculite and vermiculite filled with resin. Thus, most of the minute flaky phyllosilicate particles observed were vermiculites or vermiculitic minerals.

April and Keller (1990a, 1990b) studied mineralogy of the rhizosphere in forest soils and suggested that the pedogenic processes in the root zone differed from those operating in the bulk forest soil. In connection to this, it would be interesting to know whether or not the vermiculite particles found on root surfaces were derived from mica as *in situ* weathering products or simply accumulated by precipitation from suspension which went through root channels. There was no decisive way to determine this in our study. However, the frequent presence of aluminian vermiculite or aluminian K-depleted mica particles near and on root

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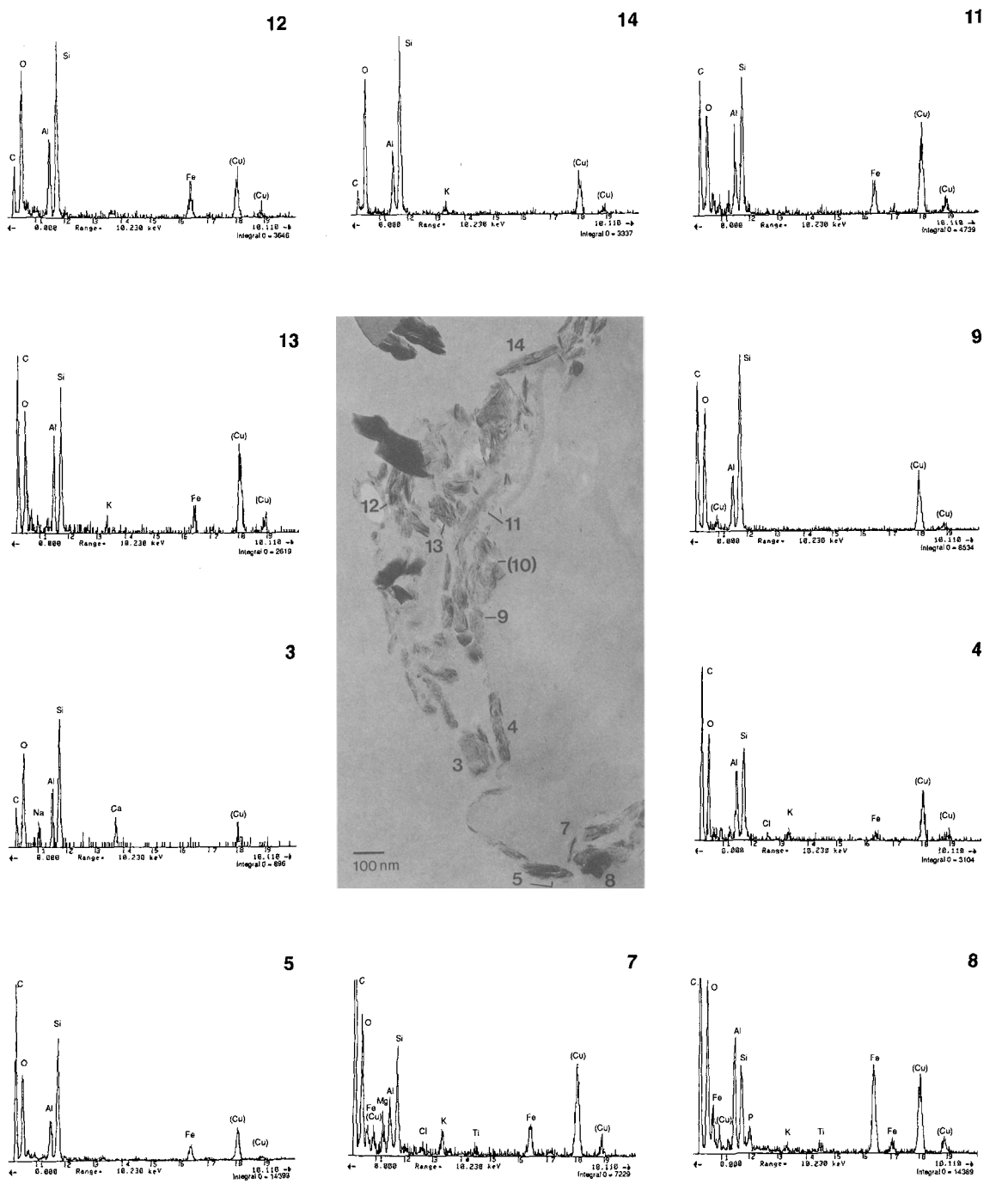

Figure 4. Transmission electron micrograph (TEM) with energy-dispersive X-ray analyses (EDX) of selected mineral particles. Numbers given in EDX charts correspond to those in TEM, respectively. Possible minerals identified for particles are as follows: (3) plagioclase; (4, 5 and 13) K-depleted mica (perhaps biotite); (7) biotite; (8) an aluminosilicate-like amphibole associated with Al-Fe oxides; (9) a dioctahedral vermiculite (aluminian); (11 and 12) aluminian vermiculite with Fe oxides; (14) K-depleted mica (illite-like).

surfaces, compared with that anticipated from the overall mineralogy of the rhizosphere, suggested that at least some of these mineral particles would have resulted from weathering on root surfaces.

Obviously, a further study is needed to characterize the nature of vermiculite and/or vermiculitic minerals on root surfaces in comparison with those in the non-rhizospheric soils. In addition, the influence of other

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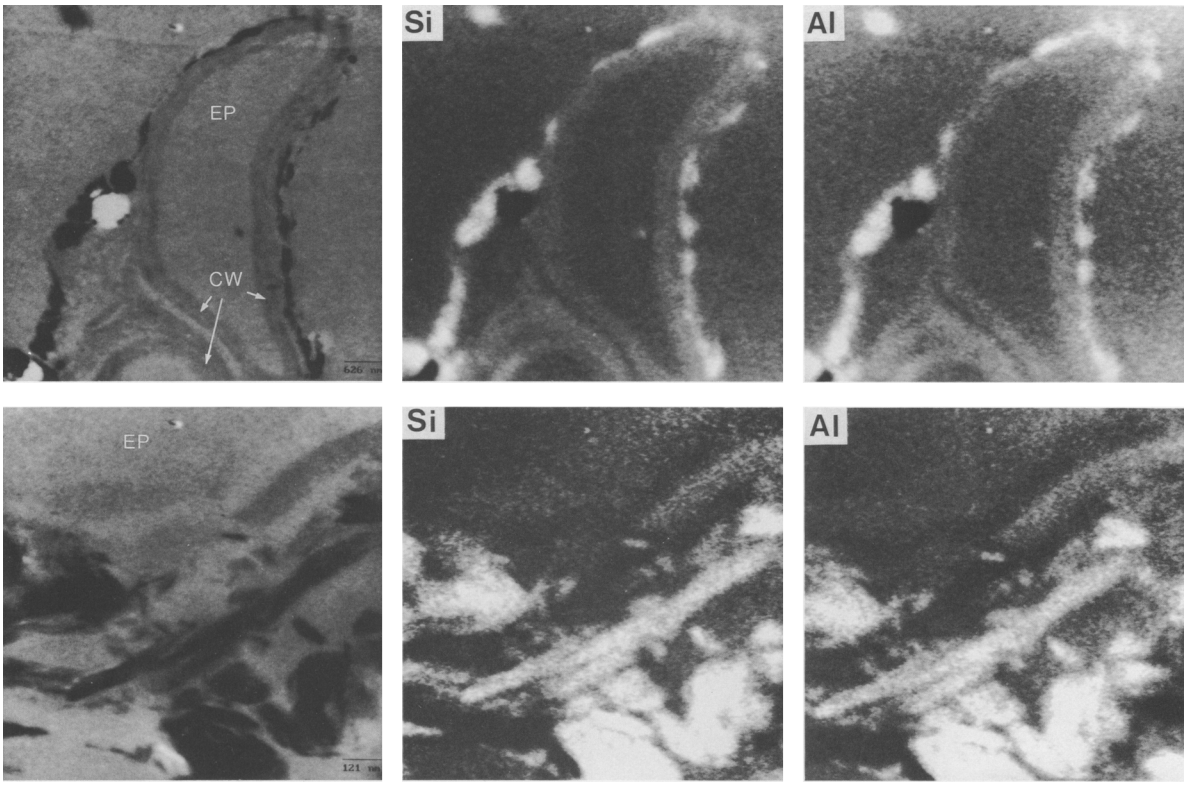

Figure 5. Transmission electron microscopy-electron energy loss spectroscopic (TEM-EELS) micrographs showing phyllosilicate particles attached on root surfaces. EP: Epidermal cell, CW: Cell wall. Scale bars indicate 626 nm at top and 121 nm at bottom, respectively.

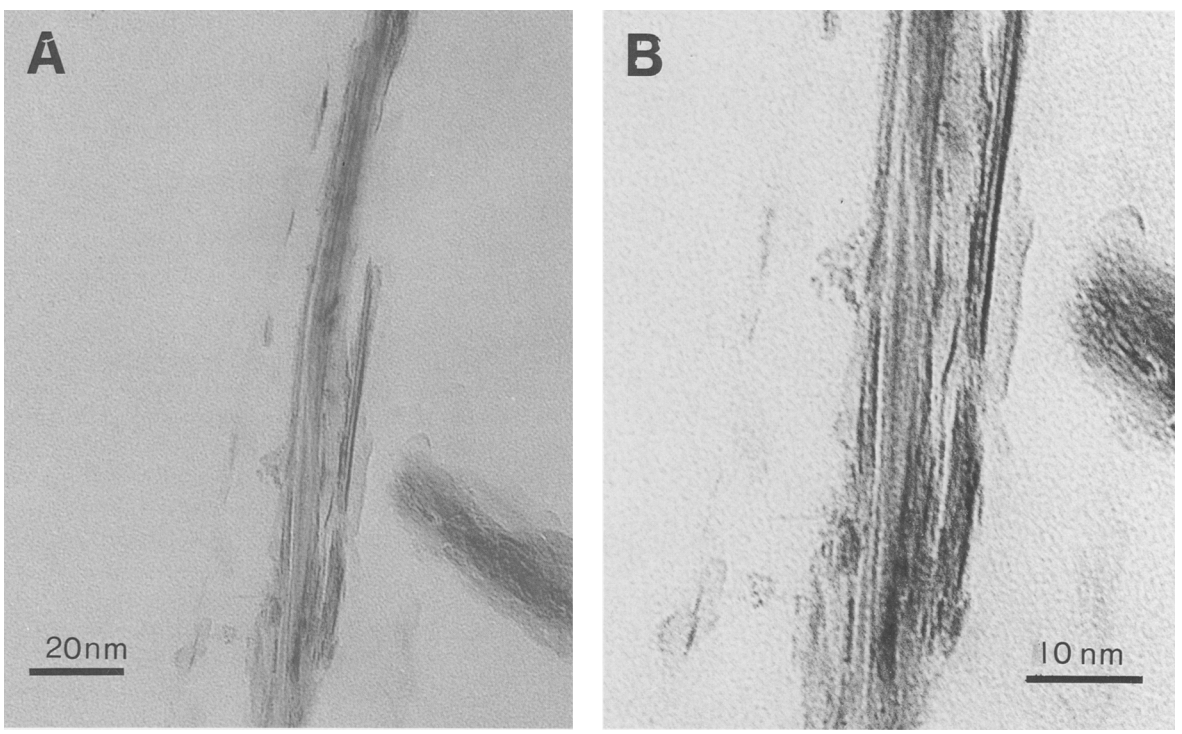

Figure 6. High-resolution transmission electron micrograph showing lattice images with variable periodicities ranging from 1.0 to 1.4 nm.

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factors such as the dispersion of these particles by plowing, should be considered.

Although the vermiculitization of mica by plant growth has been reported since the investigation of Mortland *et al* (1956), it took a long time to have the direct evidence that the vermiculitization of trioctahedral micas can take place within several weeks in the rhizosphere (Hinsinger 1993; Hinsinger and Jaillard 1993; Hinsinger *et al* 1992, 1993; Leyval *et al* 1990). Those latter reports were based on pot experiments. The results obtained here suggest a good indication that the vermiculitization of mica might have partly taken place in the rhizosphere of field grown corn.

CONCLUSION

Our findings from the present investigation may be summarized as follows: (1) mineralogy of the rhizosphere differed from that of bulk soil, (2) mineral distribution tended to be more dense in rhizospheric soil, (3) within the rhizosphere, minute platy phyllosilicate particles tended to be more concentrated near and on root surfaces, (4) the phyllosilicate particles on root surfaces were mostly vermiculitic, (5) the attachment of these particles varied along root surfaces, (6) areas in which particles were attached appeared to be areas where the mucilage had accumulated on root surfaces, and (7) the relatively high concentration of vermiculitic minerals around roots in the rhizosphere may imply that reactions with minerals near root areas are different from those anticipated from the mineral composition of bulk soils.

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