Effects of Soil Compaction on Root and Root Hair Morphology: Implications for Campsite Rehabilitation

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Abstract—Recreational use of wild lands can create areas, such as campsites, which may experience soil compaction and a decrease in vegetation cover and diversity. Plants are highly reliant on their roots' ability to uptake nutrients and water from soil. Any factors that affect the highly specialized root hairs ("feeder cells") compromise the overall health and survival of the plant. We report here initial data in our investigation of how of soil compaction affects plant roots, using the common bean as a dicot model. Soil compaction decreases overall plant growth and causes changes in root hair morphology and the F-actin cytoskeleton, critical to the function of root hairs. In addition, rates of cytoplasmic streaming, which facilitate nutrient and water uptake, are reduced in root hairs from compacted treatments. When plants were removed from compacted soils, higher amounts of total C, N and Ca were found compared to those of controls. We discuss these data in the context of rehabilitation methods in impacted wilderness areas.

Plant growth and resilience strongly depend on the ability of the roots to anchor in substratum and uptake nutrients. Nutrient uptake is facilitated by modification of the maturing root epidermal cells into specialized extensions called root hairs, which extend into the soil by tip growth and facilitate the transport of soil nutrients and microelements, as well as water, via passive and active transport across their plasma membranes. Root hair development and function are highly dynamic and restricted to a specialized zone of the maturing root. Thus, they are highly responsive to changes in the physical and chemical status of the soil.

Since root hairs grow by tip growth, conditions that affect soil pore size or produce point localized pressure may affect not only their morphology, but also their function. Tip growth in root hairs appears to be controlled by a cellular component known as actin. The polymerization of globular (G) actin monomers into filamentous (F) actin forms a highly organized network throughout the cortex of root hairs. This network is very sensitive and responsive to changes in the external environment of the cell and facilitates cytoplasmic streaming, which is critical to the uptake and distribution of nutrients to the body of the plant.

Soil compaction, bulk density and strength are important factors affecting both shoot and root growth of plants, and roots growing in soil are able to respond to changes in these soil properties to some extent (Dexter 1987). Nonetheless, plants subjected to soil compaction are more susceptible to water stress and soil-borne diseases (Smucker and Erickson 1987). Furthermore, the possible reduction in plant-associated fungi and bacteria present in the soil combined with a retardation of root hair structure and function, may result in a rapid decline of flora. Roots growing in compacted soils may also be damaged by lack of oxygen (Schumacher and Smucker 1984) and by the accumulation of toxins (Crawford 1982). In the vicinity of recreational campsites, an increase in soil compaction and a decrease in vegetation cover have been documented (Marion and Cole 1996). However, little attention has been paid, both in terms of biological mechanism and remediation, to the changes that occur in the morphology and physiology of dicot roots. These data are crucial not only to monitoring and assessment of impacts, but in prescribing methodologies for rehabilitation of impacted areas. This is especially true since user impacts may vary depending on the type of soil, the diversity (types) of vegetation cover and the general features of terrain. Thus, the prescription of generalized assessment and rehabilitation protocols for heavily impacted campsites may not be effective in all areas.

Materials and Methods

Soil and Planting Conditions

Experiments were conducted using a sandy loam soil (approximately 67% sand, 23% silt and 10% clay) in a rooftop greenhouse with an automatic drip water system modified to an area sprinkler system. Soil was placed in plastic cylinders approximately 14 cm in diameter, and four treatments were applied: 1) no compaction 2) light compaction (~0-2.5 MPa) 3) medium compaction (~2.6-4.5 MPa) and 4) heavy compaction (~4.6-6.0 MPa).

Compaction and Penetrometer Resistance

Compaction was achieved using a 15 kg weight and assessed using a Haughn penetrometer. Soil penetrometer resistance was measured twice for pots, before and after the plant growth experiment. Additional measurements of penetrometer resistance were made on each pot at the time of plant harvest. A hand-held penetrometer equipped with a conical steel probe of 2.5 mm in diameter (with relieved

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shaft) was used in all measurements. During soil penetration of the probe, mass equivalence of force (M, kg) for each cylinder was determined using an electronic balance with resolution 5 g. Values of M were recorded to a soil depth of 75 mm, and maximum values of penetrometer resistance (Mpa) were estimated from the maximum values of M according to the equation $P = 4\ Mg/pd^2$.

Planting and Growth

Seeds of common bean (*Phaseolus vulgaris* L. var. Vista) were sterilized using a weak solution of sodium hypochlorite (10%) in distilled water and dried under sterile conditions. One seed was planted per cylinder to eliminate artifacts that may have occurred due to competition from neighbouring roots. Seeds were covered with approximately 4 mm of sandy loam soil and gently packed. In each of the six replicates, 20 cylinders were used. Five were used as controls, and five pots were used for each treatment (low, medium and high compaction). After planting, pots were placed on a perforated metal sheet on a 3 x 1 m planting table equipped with drains. Temperature was controlled by automatic windows and vents, as well as automatic heat lamps. Measurements of temperature indicated that temperatures remained relatively constant at 24 degrees C during the day and 14 degrees C during the night. Soils were not supplemented with fertilizers.

Plant Root Analysis

Seedlings were harvested 21 days after planting. The entire soil column was removed from the cylinder, and plants were gently separated out and laid flat on a white plastic tray. Soil adhering to roots was removed as much as possible by gentle washing with lukewarm distilled water. The root system of each seedling was examined under a Leitz AM443 dissecting microscope to obtain quantitative measurements of numbers of lateral roots, lateral root length and primary root length. Qualitative assessment of root hairs was made under a Zeiss Axiophot microscope equipped with DIC optics, and digital images were captured using a top-mounted Zeiss CCD camera.

Staining for F-actin (Cytoskeleton)

Coverslips were affixed to plant roots so that some root hairs lay flat against the glass. Root hairs were then permeabilized by incubating them with 0.01% w/v (0.1 mg/ml) saponin in distilled water for one hour. They were then rinsed and labeled with rhodamine phalloidin (RP), a fluorescent probe that specifically binds F-actin, by placing 100 □L of 6.6×10^{-7} M RP. A stock solution of 6.6×10^{-6} M RP was prepared in methanol and stored at –20 °C. Prior to staining, the RP was desiccated and reconstituted in ASW to produce a working solution of 6.6×10^{-7} M. Root hairs were incubated in RP for at least 12 hours. Immediately prior to microscopic observations, cells were rinsed for one hour in distilled water. Controls for the specificity of RP binding were conducted by incubating root hair cells with an excess of unlabeled phallacidin (2×10^{-6} M) in ASW for six hours prior to staining with RP. Phallacidin has higher specificity than RP for F-actin and therefore should greatly reduce or eliminate RP staining. All procedures involving staining for F-actin were done at 23 °C.

Microscopy

Rhodamine phalloidin-labeled root hairs were observed on a Zeiss comound microscope equipped with epifluorescence using a narrow bandpass emission filter (605 ± 27 nm band width filter; Chroma Technology, Brattleboro, VT).

Analysis of Root Exudation

For analysis of rood exudation, plants were carefully removed from pots and the remaining soil carefully examined for plant organic debris using a dissecting microscope. Five g of soil was mixed with 100 ml of distilled water and mechanically shaken for 20 minutes. For N and Ca, the supernatant (liquid fraction) was used; for C, dry soil was used. Total nitrogen was assayed by Kjeldahl digestion and ammonium analysis using standard Autoanalyzer techniques (Technicon Industrial Systems). Cation analyses were performed on a Perkin-Elmer Atomic Absorption Spectrophotometer. Calcium and carbon were determined with furnace and flame analysis, respectively, under optimized conditions. Changes in pH were determined by using a hand-held pH indicator under constant conditions of moisture and temperature.

Results

Effects of Compaction on Primary and Lateral Roots

A representative of seeds grown under control (no compaction) conditions is shown in fig. 1A. Overall morphological differences between control plants and those grown under moderately compacted conditions (approximately 4 MPa) consisted of the following, generally conserved, features for compacted treatments: plants were 1) shorter (as measured from soil surface to apical tip), 2) possessed reduced leaf surface area and 3) rarely possessed straight stems (see fig. 1B). With respect to root morphology, increasing compaction (low>medium>high) resulted in an increase in the length of the primary root and a corresponding decrease in the number of lateral roots. This inverse relationship varied from replicate to replicate but followed a fairly consistent pattern (fig. 2). Sub-apical swelling was observed in most primary and lateral roots (n=112), regardless of their average distal diameter. Diameters of lateral root tips were of at least half of those of primary root tips. A representative root tip showing the morphology of typical sub-apical swelling is shown in fig. 3A. Root tip squashes revealed that an increase in the thickness of the cortex, but not the stele, contributed to the increase in sub-apical root tip diameter (not shown). It was also observed that the root cap appeared to be consistently thicker; however, this potential morphological effect of compaction was not quantified. Since none of the plant roots had reached the bottom of the cylinder, there were no plants that were considered to be experiencing artifacts from impedance other than soil strength. Increasing soil compaction resulted in a

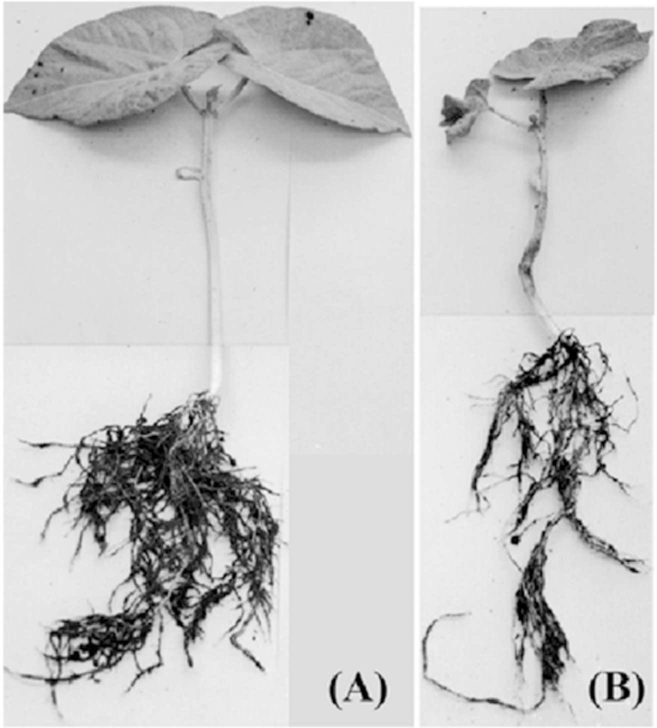

Figure 1—Representative control plant (A) vs. plant grown in compacted soil (approximately 4 MPa), (B). Compaction resulted in plants with decreased leaf surface areas and lower dry weights, as well as longer primary roots and fewer lateral roots.

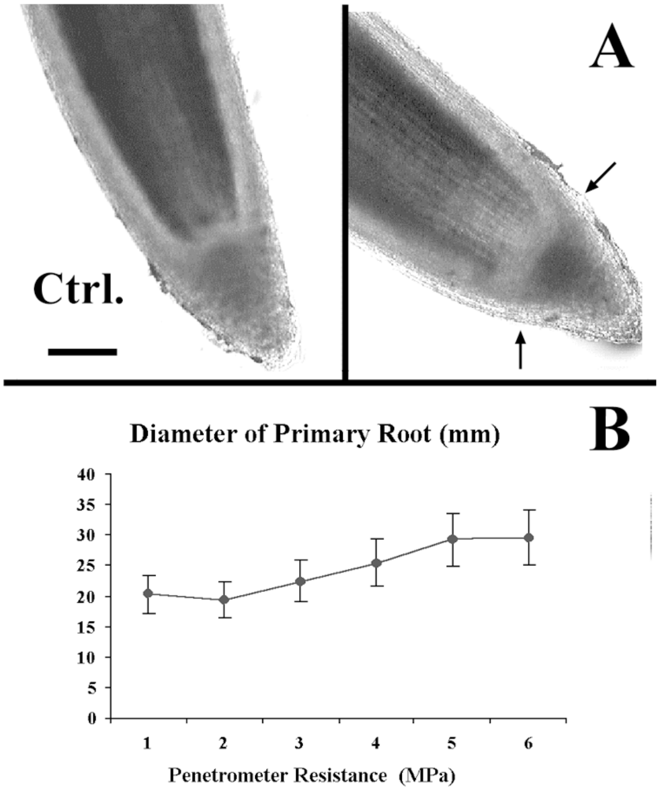

Figure 3—A. Micrograph of representative control (left) and compacted root tip (right). Sub-apical swelling is apparent in the latter (arrows), and the overall root diameter is increased. Bar=2 mm. B. Graph demonstrating an increase in primary root diameter with increasing soil compaction.

proportional increase in sub-apical swelling of the primary root tip (fig. 3B), in addition to the observed increase in primary root length.

Effects of Compaction on Root Hairs

Root hairs were examined under a compound microscope equipped with differential interference contrast (DIC) optics in order to obtain a clearer image of these extremely fine cells. Roots hairs observed in roots from control plants appeared as shown in fig. 4A, left image. Overall length to width ratios were on the order of approximately 22:1 in controls and approximately 3:1 for those observed from moderately compacted roots (fig. 4B, left image). Cytoplasmic streaming rates were measured from videotaped analysis (table 1) and decreased proportionately with increasing soil compaction. To investigate the status of the filamentous actin network, root hairs were permeabilized with saponin and labelled with rhodamine phalloidin (fig. 4A and B, right images). The F-actin cytoskeletal network in root hairs from control plants consisted of bundled filaments that ran parallel to the long axis of the cell (fig. 4A, right image). Cytoplasmic streaming occurred along these bundles at high rates (see table 1) throughout the cell. Comparatively, root hairs from plants grown in moderately compacted soil possessed an F-actin network that appeared disorganized. Bundles often were observed to terminate along various points (fig. 4B, right image), and cytoplasmic streaming occurred sporadically, both spatially and temporally, in association with the fragmented cytoskeletal array. As soil compaction increased, the length of root hairs decreased, and the overall width (diameter) increased (fig. 5). Few root hairs with a decreased length to width ratio possessed

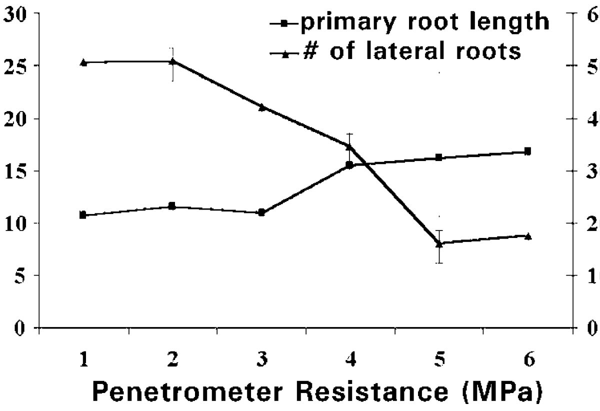

Figure 2—Graph showing inverse relationship between primary root length and number of lateral roots. As primary root length increases, the number of lateral roots decreases.

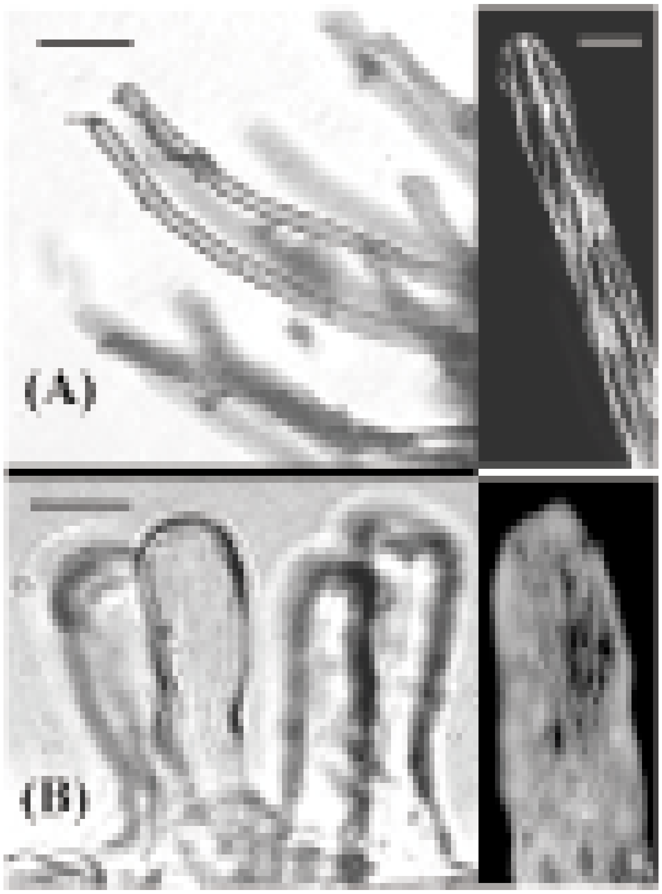

Figure 4—Left image. Root hair morphology in control plant roots (A). Root hairs possess a high surface area to volume ratio (longer lengths and smaller diameters), whereas root hairs from treated plants possess a smaller surface area to volume ratio (B, left image). Right images: fluorescence micrographs showing the F-actin cytoskeleton of root hairs. F-actin in control root hairs has well-defined bundles, oriented to the long axis of the cell (A, right image). F-actin in treated root hairs exhibits signs of disorganization (B, right image). A, left image, Bar=1 mm, right image, Bar=200 mm. B, Bar=750 mm.

F-actin arrays that resembled controls and supported continuous, vigourous cytoplasmic streaming.

Root Exudation

In order to investigate whether compaction resulted in increased root exudation, carbon (C), nitrogen (N) and calcium (Ca) present in soils were measured after seedlings had been removed. Compaction resulted in an increase in C, N and Ca present in the soil of pots after seedlings were removed. These values were expressed as a percent increase from controls. A decrease in pH was also noted in soils subjected to moderate and heavy, but not mild compaction. These data are summarized in table 2.

Discussion

Soil compaction, whether caused by trampling or impact from machines, has adverse effects on plant health. Compaction may result in 1) an increase in bulk density, 2) the elimination or decrease of biologically available pore space (into which fine root processes may extend) (Kooistra and others 1992), 3) a change in soil gas balance and changes in soil moisture status and regulation (Kuss 1986). These effects can be linked to morphological and physiological changes at the level of the plant root. Morphological changes appear to include restriction of root extension and shoot growth, as well as modifications of the root pattern and root diameter (Ikeda and others 1997).

Table 1—Effects of compaction on rates of cytoplasmic streaming in root hairs.

Compaction^a	**Rate of cytoplasmic streaming (□m/min)**
CONTROL	22.5 ± 5.7
MILD	20.8 ± 9.4
MODERATE	14.2 ± 2.7
HEAVY	9.9 ± 6.1

[a] Control: no compaction; mild: 1-2 MPa; moderate: 2.1-3.45 MPa; heavy: 3.5-5.0 MPa.

The impacts of camping on the status of vegetation cover and soil structure and function may be severe (Hammitt and Cole 1987) and almost always involve soil compaction (Marion and Cole 1996). Often, the relationship between campsite use and impact is positive but nonlinear due to differences in soil structure, vegetation diversity and abiotic factors such as hydraulic balance and topography. Thus, rehabilitation methods that focus on mechanical but not biological reparation may not overcome impact-induced biological deficits such as changes in root function and loss of microbial biomass and the sustenance of initial natural recovery processes such as vegetation succession (Wardle 1992).

Primary and Lateral Root Systems

Our data are consistent with those reported by Pietola and Smucker (1998), in that compaction actually increased

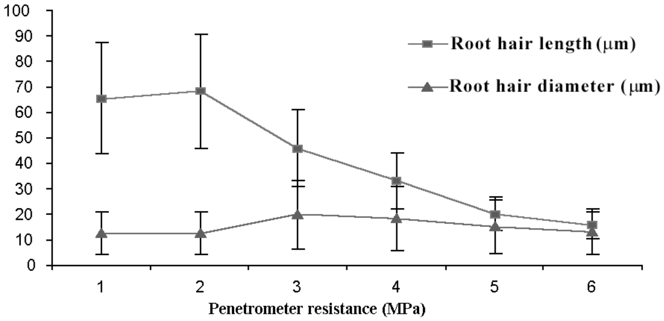

Figure 5—Graph showing changes in root hair length and diameter of *P. vulgaris* grown in compacted soils. Root hair length decreases, and diameter increases. Cytoplasmic streaming in these cells is greatly reduced (see table 1), and the F-actin cytoskeleton becomes disorganized (see fig. 4). $p<0.05$.

Table 2—Effect of compaction on total carbon (C), nitrogen (N) and Calcium (Ca) present in soils.

Compaction[a]	Total C[b]	Total N[c]	Total Ca[d]	pH
CONTROL	0.0	0.0	0.0	7.06 ± .25
MILD	9.7 ± 0.3	8.2 ± 0.9	*N.D.*	7.23 ± 0.11
MODERATE	12.5 ± 1.4	11.3 ± 0.7	2.3 ± 0.8	6.8 ± 0.17
HEAVY	32.8 ± 6.9	29.2 ± 1.3	10.1 ± 1.4	6.6 ± 0.15

[a] Control: no compaction; mild: 1-2 MPa; moderate: 2.1-3.45 MPa; heavy: 3.5-5.0 MPa.

[b,c,d] Reported as percent increase from control.

primary root length, but they are contrary to reports that describe a decrease of primary root lengths of other plants grown in compacted soils (Atwell 1988). The data also suggest that soil compaction that results in a change in lateral root number, as well as root hair function may have greater effect on overall plant health than when primary root growth alone is inhibited in deeper soil layers (Goodman and Ennos 1999). Thus, plants may respond to changes in soil structure (compaction) that are fairly mild to moderate (compaction of the topmost layer of soil). They also imply that rehabilitation of sites that have been stripped of topsoil layers, thus exposing mineral soil, may be far more challenging and complex than that of sites which are only moderately impacted.

Roots Hairs

Our data show that, in the system studied, structure and function of root hairs are affected by soil compaction (fig. 4). In young roots, the epidermal root hairs, which greatly increase the absorbing surface area of the root, absorb water and minerals. Root hairs are relatively short- lived and may reach maturity within hours. The production of new root hairs occurs just beyond the region of elongation and at approximately the same rate as that at which the older root hairs are dying off at the upper end of the root hair zone. Any factors that compromise the structure and function of these cells will affect the status of overall plant health. Since these cells are relatively short-lived and generated quite rapidly, seedlings germinated on soil that has not been adequately de-compacted may be unable to adequately anchor in soil and uptake nutrients and water. Furthermore, epidermal cells of the root produce a mucigel that enables the root hairs to establish close physical contact with soil particles (Ulehlova and others 1988). If soils are de-compacted so that large air spaces are present, this contact, critical to uptake, may be greatly reduced. This mucigel is also hypothesized to have other functions such as facilitating carbon sequestering near the root, facilitating the passage of root processes through soil (Ulehlova and others 1988) and attracting soil microorganisms to the vicinity of the root (Ikeda and others 1997).

Exudation

An increase in root exudates from maize and cereal roots grown in compacted soils has been reported by Boeuf-Tremblay and others (1995) and Barber and Gunn (1974), respectively. Root exudation may be described as a generalized stress response to conditions, for example, where the root physical structure has been compromised or where a toxicity response has been initiated such as in plants grown in low pH soils containing aluminum (Taylor 1995).

Our data are consistent with those of Boeuf-Trembly (1995) and Barber and Gunn (1974), in that compaction increased the production of root mucilage, which contributed to an increase in total C, N and Ca levels of soils surrounding the root. The function of increased root exudation may include, but not be limited to, 1) chelating toxic compounds by changing the localized pH (as appeared to be the case for aluminum toxicity) and 2) providing an environment which favours the aggregation of culturable bacteria (Ikeda and others 1997). This latter function has interesting implications for plants growing under compacted soil conditions and for seedlings introduced to sites for rehabilitation. Soil compaction may not only affect plant growth, it may also affect the diversity and numbers of soil micro-organisms (Zabinski and Gannon 1997). The functions of these diverse organisms are poorly understood; however, they may function in roles such as nitrogen fixation, the decomposition of debris, the stimulation of root growth and the accumulation of nutrients in the vicinity of the maturing root (Perry and Amaranthus 1990; Turkington and others 1988). Zabinski and Gannon (1997) report that bacterial and fungal components of the soil community were severely disrupted in soil from campsites. Thus, plants growing on increasingly compacted soils, which may be increasing root exudates in order to attract micro-organisms, may be unable to do so due to the absence or decline of the latter. A caveat, however, is that soil micro-organisms may be highly dependent on the presence of vegetation, which may provide a carbon source for substrate utilization (Rovira 1995). Unfortunately, our understanding of the dynamics of the plant root-soil microorganism relationship is extremely limited, so our ability to speculate on the nature of this, possibly nonlinear, biology is severely restricted.

Implications for Rehabilitation of Recreation Wilderness

Although preliminary, our data provide evidence that moderate soil compaction affects the structure and functioning of a dicot plant root system. These data provide evidence for a possible mechanism by which compaction of soils in the area of campsites may cause a decrease in vegetation cover. Thus, in areas where vegetation is partially removed, remediation efforts that involve the introduction of nutrients to the impacted area (such as the raking of humic soils and detritus) after mechanical de-compacting of soils may not effective. Not only are root hairs unable to uptake nutrients, as is suggested by a decrease in cytoplasmic streaming, the plant root exhibits increased exudation of organic N, C and Ca-containing compounds, possibly as a generalized stress response (Dexter 1987) or as a mechanism to attract root-associated, culturable bacteria (Barber and Gun 1974; Boeuf-Tremblay and others 1995). We are particularly interested in the changes that occur in the root hairs due to soil compaction. If these cells do not function optimally, plant growth will be compromised even if seeds germinate in disturbed areas. Thus, monitoring the status of root hairs has implications for predicting the effectiveness of rehabilitation in impacted areas and may be accomplished easily, with minimal gear and time. Mechanical loosening of soils may be beneficial in attempting to reestablish a functional soil matrix; however, outcomes other than the ideal "ratio" of soil aggregate to pore size may occur. These include, but are not restricted to, 1) loosening of only the top few centimetres of soil with sub-layers remaining compacted, 2) over-loosening of soil such that root-soil contact is decreased, 3) loosening of soil so that aggregates consist of large, compacted pieces of soil with large air spaces between them.

Ongoing Research

Currently, we are extending and bifurcating the study to 1) an investigation of dicot roots of woody and nonwoody plants from soils in sites that have been compacted by foot and livestock traffic, as well as the behaviour of root processes in de-compacted field plots that have been re-seeded and planted in an attempt to restore vegetation cover and, 2) a study involving rehabilitation methods that transfer soil normal flora from the perimeter of heavily used sites to the impacted area in order to facilitate seedling growth. The effects of soil compaction on root structure and function are complex. Here, we report data for a specific type of soil and dicot species. Compaction effects may vary depending on soil type, soil moisture status, plant type, etc. Thus, a great deal of research is needed to identify the potentially common mechanisms that may underlie varying responses to soil compaction.

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