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微根窗技术及其在植物根系研究中的应用

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摘要: 植物根系对固定植株和获得水分和养分起重要作用, 但是土壤不可观测性的限制, 给根系生态学的研究带来一定的困难。因此, 找到原位观察根系生长的方法对研究根系生态学就显得尤为重要。目前微根窗技术被认为是研究根系生态学最有前途的方法。从微根窗系统的组成、微根窗管的安装、微根窗图象的收集及微根窗数据的利用等几个方面进行了概述。阐述了在微根窗使用和操作过程中需要注意的几个问题, 微根窗管与土壤之间的良好接触是获得高质量微根窗图像数据的前提和基础; 图象收集的频率依赖于测定和计算的根系参数, 如果想得到根系现存量、生产力、更新和寿命的信息, 必须避免采样间隔时间过长。

关键词: 微根窗; 根系生产; 根系周转; 根系死亡

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Applications of minirhizotron techniques to root ecology research

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Abstract: Plant root plays an important role in anchorage and water and nutrient acquirement. However methodological limitations have troubled many researchers because of the inaccessibility of belowground systems. It appears important to find a good approach for *in situ* observation of root growth. The minirhizotron method has been regarded as the most promising method for root studies so far. This article aims to introduce the method to researchers in China and to synthesize several issues involved in the application of the minirhizotron method in terms of it's hardware, installation, digital image collection, data acquisition, and subsequent data analysis.

Minirhizotron tubes are main parts of the minirhizatron system. Minirhizotron tubes of different materials make different influence on growth and longevity of plant roots. Glass is the material carrying minimal influence at present. A key requirement for installation of minirhizotron tubes is to insure a good contact between the tube surface and the surrounding soil. Minirhizotron installation usually adopts a two-step tube installation method. But this method may not operate very well in several kinds of soils such as heavy textured soils, soils with abundant rocks and cobbles, soils with argillic (clayey) horizons, or loose structureless soils. The angles of minirhizotron installation are commonly 30° or 45° off the vertical.

Frequency of minirhizotron image collection has marked influence on the calculated veracity of the growth and death of roots during sampling intervals, for the growth and death of root occur frequently. The frequency of image collection depends upon the root demography and the time and resources available. If information about root standing crop, production, turnover and lifespan is desired, long sampling intervals should be avoided.

The basic information of birth, death, root number and root length can be derived from the minirhizotron image, which make it much easier to calculate root production and turnover (mortality). Universally, it is based on the changes of color and morphology to identify living roots or dead roots. If the root changes from white to brown or black, or the root becomes shrivelling, softening, partial decomposed, or disappeared, it is viewed as dead root, but not all the roots that have changed from white to brown are dead roots. So it is still difficult to accurately discriminate the living roots and dead roots only through

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minirhizotron image.

Key words: minirhizotron; fine root; production; turnover; mortality

植物根系不但有固定植株、吸收水分和养分的作用, 而且根系生产和周转直接影响陆地生态系统碳和氮的生物地球化学循环[1, 2]。虽然都清楚地认识到植物根系的功能和作用, 但由于受根系研究方法的限制, 直到目前对陆地生态系统根系生态学的研究还很薄弱[3], 因此寻找并建立新的根系研究方法就显得至关重要。由于受土壤不可观测性的限制, 根系研究方法进展缓慢, 传统的研究方法如挖掘法[4]、剖面法[5]、盆栽法[5]及土柱法[6~8]仍在大量使用。近年来随着光学和电子学的发展, 特别是微根窗法(Minirhizotron)的应用, 使根系生态学的研究得到了较快的发展。

当前, 在根系生产力及其周转方面的研究中, 改进的微根窗技术很有前途[9, 10]。它是唯一提供多个时间段内原位重复观测根系的方法, 这种测量方法是非破坏性的, 是传统的研究方法不可替代的[10, 11]。所以, 微根窗技术被广泛应用于根系动态及其功能的研究[12, 13]。目前, 在国外, 微根窗技术在森林[14, 15]、果园[16, 17]、草地[18]、沙漠[19, 20]和农业生态系统[21, 22]等植物根系研究中已经普遍应用。但是, 由于技术设备和其它一些因素的限制, 微根窗技术在我国的利用受到一定的影响。因此, 从微根窗系统的组成、微根窗管的安装、微根窗图象的采集及微根窗数据的利用等几个方面进行概述, 以期为微根窗技术在我国广泛应用提供一定的指导。

1　微根窗系统的组成

自Bates[23]首次提出微根窗概念后, 微根窗就成为了解根系形成和功能的一个常用工具。Brown 和Upchurch[24]对微根窗的定义: "一个微根窗系统是由多组分组成的, 在土壤中真实地或通过图象记录了一般难以接近的植物根系生长情况"。利用微根窗系统观察根系的工具主要有: 根系潜望镜[25]、内窥镜[18]、光学孔径检查仪[26, 27]、光学照相机[28]、照明镜[29]以及微型彩色摄像机[9, 14]等。早期的微根窗研究比较简单, 收集到的数据量少, 随着光学和电子学的发展, 从微根窗收集到的数据和信息量大幅度增加, 使微根窗技术得到了长足的发展。目前, 一个典型的微根窗系统是由一个插入土壤中的微根窗管、摄像机、标定手柄、控制器和一台计算机组成(图 1)[30~32]。

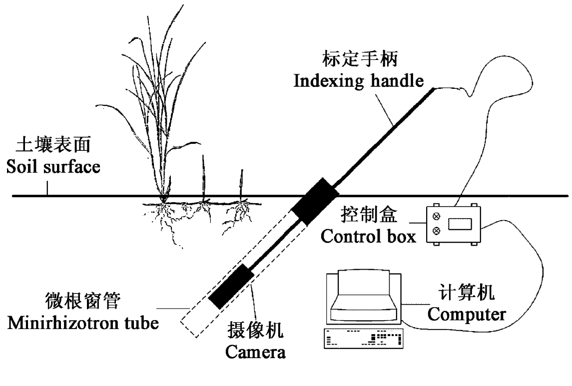

图 1　微根窗图象采集系统[30, 32]

Fig. 1　Minirhizotron image acquisition system[30, 32]

2　微根窗管的安装

微根窗管是微根窗系统的一个重要组成部分, 一般是透明的玻璃或塑料管。管子的形状通常是圆形的[24], 但也有方形管[18]。膨胀管[33]或加压管[34]也通常被使用。微根窗管一般由树脂玻璃、丁酸盐纤维素(CAB)、聚碳酸脂、丙烯酸树脂和玻璃制成。对材料的选择基于成本和实用性。有些研究报道这些材料对根系生长情况没有影响[24, 32, 35], 但Withington 等[27]比较了玻璃、丙烯酸树脂和丁酸盐纤维素管对苹果(*Malus domestica*)、阔叶树、松树根系形态和生长动态的影响, 发现这些材料在有些植物中对根系生长影响较小但对根系的寿命影响比较大, 影响根系寿命最大的材料是丁酸盐纤维素, 同时指出在这些材料中玻璃对植物根系的生长和寿命影响最小。微根窗管必须是密封的, 地上部是绝缘的, 并将其涂成黑色以减少管子的热量传导, 同时盖上不透光的盖, 避免光的射入影响根系生长[36]。微根窗管的安装应保证对管子周围的土壤扰动最小[37], 其次应使土壤与微根窗管之间的接触紧密, 如果土壤与管壁没有良好的接触, 土壤中根系的生长就不具有代表性[10]。传统的安装方法通常是用钻钻一个深度和大小与微根窗管直径相符的孔, 然后将管子插入孔中。这种简单的安装方法一般效果都很好[38~40]。有些研究采用双步骤安装方法, 即先用一个直径略小于微根窗管直径的螺旋钻移出大部分的土壤, 之后用一个锥形钻头切出最终符合管直径大小要求的孔, 并在插入管子之前用圆形的细金属刷子在孔内刷出条纹[41, 42]。显然土壤类型影响微根窗管安装方法的采用, 在质地较重的土壤、含有石块的土壤或松散无结构的沙土上用传统方法安装微根窗管有一定的问题[13], Phillips 等[20]报道使用气压岩石钻孔机安装微根窗管可以很好地解决在上述土壤中用传统方法安装微根窗管存在的问题。Wilcox 等[43]应用了气压岩石钻孔机在莫哈韦沙漠安装微根窗管研究了四种沙漠灌木根系生长动态和土壤水分的关系。微根窗管安装的角度范围比较大, 可水平、垂直或成 22 角、30 角、35 角、45 角、60 角等非垂直角度放置在土壤中。成角度放置的微根窗管在获得有关根系垂直分布方面的信息要优于水平放置的微根窗管, 并且在野外安装时, 成角度放置的微根窗管比水平放置的微根窗管更易安装[32]。根据文献资料分析, 微根窗管安装大多数是与垂直方向成 30 或 45 角(表 1)。微根窗管的安装角度可能影响根系的生长[29], 但关于这方面的报道非常少。

3 微根窗图象的采集

微根窗图象采集一般是使用微根窗系统的摄像机把图象收集到录像带里[43, 56]。为了确保在微根窗管上同一地点重复取样,通常在微根窗管上刻上标记或使用带有刻标定位的自动手柄[39, 40, 43, 56, 57, 67, 71, 73, 74]。微根窗图象采集的频率依赖于测定的根系参数,图象采集的时间间隔从文献资料看有 1 周、2 周、4 周或更长时间 6～16 周的都有[14, 15, 59, 62, 75]。一般在冬季采样次数减少[55]或不采样[56, 57]。微根窗图象采集频率对计算根系生长和死亡的准确性影响很大,因为在微根窗采样间隔期内常常出现根系形成和死亡,所以较长的采样间隔会低估根系的生长和死亡率[13, 32, 65]。Dubach & Russelle[44]评价了采样频率对根系形成和死亡数据准确性估计的影响,他们设置了从 1 到 8 周不同的采样间隔,得出紫花苜蓿(*Medicago sativa* L.)在 2 周或 3 周的采样间隔内计算的根系周转率的准确率为 97%;采样的时间间隔为 8 周时,计算的根系周转率的准确率只有 75%(与采样间隔 1 周相比)。同时他们比较了相同的采样时间对紫花苜蓿和百脉根(*Lotus corniculatus* L.)根系死亡率的影响,发现采样间隔同为 8 周时对紫花苜蓿根系死亡率的估计少 25%,而对百脉根根系死亡率的估计只少 1.3%以下,原因是百脉根根系存活时间比紫花苜蓿长,因此不同植物种类有不同的适宜采样时间间隔。Johnson 等为评价采样间隔对根系生产力和死亡的影响,1997 年在美国俄勒冈州研究了花旗松(*Pseudotsuga menziesii*(Mirb.))的根系生产力。指出当采样间隔增加到 4 周时,对根系生产力的估计大约比采样间隔为 2 周时降低 28%,当采样间隔进一步增加到 8 周时,对根系生产力的估计大约降低 54%。根系死亡率显示出同样的趋势,另外采样时间间隔对根的数量变化影响不如根长敏感[32]。因此选择合适的采样间隔是非常重要的,因为它不但影响着微根窗根系数据的质量,而且它还决定着工作量和资金的投入。

表 1 植物根系研究中微根窗角度利用的频次

Table 1 Frequency of minirhizotron tube angle used in fine root studies

管安装角度 Angle of tube installation	次数 Count	占百分比(%) Percent of total	参考文献 Reference
0.0	1	2.6	[44]
15.0	1	2.6	[45]
21.0	1	2.6	[46]
22.5	1	2.6	[47]
28.3	1	2.6	[48]
30.0	10	25.6	[18][34][49][50][16][51][19][20][52][17]
35.0	1	2.6	[24]
40.0	1	2.6	[53]
45.0	16	41.0	[54, 55][56][57][13][58][59, 60][61, 62][40][63][64][65][66][67]
60.0	3	7.6	[68, 69][70]
90.0	3	7.6	[29][71][72]
Totals	39	100	

4 微根窗数据的利用

从微根窗图象中获得根系数据和信息或者将微根窗图象数字化通常采用两种方法,一种用手工描绘[10, 76];另一种是使用计算机软件,目前使用比较多的软件有美国杜克大学生产的"RooTracker"根系分析软件[43, 67, 77]和美国密歇根州州立大学生产的"ROOTS"根系分析软件[2, 56, 63, 64]。在微根窗图象中可以得到根的出生、死亡以及根系数量和根系长度等信息,也可以根据总微根窗画面面积来确定总根长,所以这些二维空间观测数据也能反映区域根长密度($cm \cdot cm^{-2}$)。同样,可以利用观测的根长和直径乘积计算根面积,类似地有时也可以计算根面积密度($cm^2 \cdot cm^{-2}$)[37]。根系数量对采样间隔不敏感,所以一些研究利用根系数量来评价根系生产力和周转[19, 68, 78]。但是利用根系数量来估计根系生产力和死亡率时,其值一般都偏低,因为它未能说明根系的伸长生长和现有根系的长度损失[32]。利用根系长度可以较好确定根系生产和死亡,但必须注意确保采样间隔足够短,Johnson 等[32]报道采样间隔在 2 周以内可以保障对根系生产力和死亡率估计的准确性。为了更好地描述生态系统过程和与其它测量方法进行比较,在利用微根窗评价根系现存量、生产力和周转时,常将面积单位转换成体积单位。Merrill & Upchurch[50]提出了一个计算单位体积根长密度的公式,计算方程为 $RLD_v = E_f N_{ob} / A_{ob}$,这里 RLD_v 是按体积计算的根长密度($m \cdot m^{-3}$);N_{ob} 为测定的根数;A_{ob} 为测定的微根窗的面积(m^{-2});E_f 为理论转换因素($m \cdot m^{-1}$)。也可以使用另一个方程计算每单位体积的根长密度,其计算公式为 $RLD_v = L / A \times DOF$,RLD_v 是按体积计算的根长密度($m \cdot m^{-3}$),L 是微根窗画面观察的根系长度(m),A 是微根窗观察的面积(m^2),DOF 是微根窗管到周围土壤的距离(m)。其中 DOF 的取值范围一般是 2～3mm[26, 28, 79, 80]。如果知道确切的根长或每克根系生物量的根长,那么按体积计算的根长密度可以转换成生物量密度。计算方法为 $RBD_v = \sum_i \frac{RLD_i}{SRL_i}$,这里 RBD_v 是按体积计算的根系生物量密度($g \cdot m^{-3}$);RLD_i 指第 i 级根系直径的体积根长密度($m \cdot m^{-3}$);SRL_i 指第 i 级根系直径的根长($m \cdot g^{-1}$)[32]。微根窗的主要优势之一是可以追踪单个根的生长和死亡,因此可以很容易估算每个时间间隔内根系的生产和周转率[13, 81]。对微根窗图象内活根和死根的鉴别还是非常困难的,鉴别死根一般通过颜色和形态的变化,根系颜色由白色变为褐色、黑色或根系形态出现枯萎、腐烂、消失的被确认为是死根[2, 82],但是有时即使是颜色由白色变为褐色的根也不都是死根[83],所以有些研究者只将出现腐烂或消失的根确定为死根[67]。

尽管利用微根窗技术研究根系生态学具有不可比拟的优点,但是它也存在一定的局限性和缺点。手工图象分析需要大量时

间和工作量, 虽然自动化图象分析可以迅速地提取数据, 但是当前的软件还不能很精确地鉴别死根。如何使微根窗及其数据使用的范围更广阔, 也是需要进一步解决的问题。另外, 在使用微根窗技术时还应注意下面的问题, 在质地疏松的土壤, 安装微根窗管的关键和必须注意的是确保土壤与微根窗管之间的良好接触。同时在微根窗管安装和图象采集之间要有一定时间的等待期, 以保证根系在微根窗周围的空间定植和营养水平恢复到扰动前的水平[20, 32]。图象采集的频率依赖于测定和计算的根系参数, 如果想得到根系现存量、生产力、更新和寿命等信息, 必须避免采样间隔时间过长。

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