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土壤磷形态组分分级及 ^{31}P-NMR 技术应用研究进展

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摘要: 农田生态系统中土壤磷形态转化，影响土壤磷对作物的有效供应。土壤磷分为无机磷和有机磷两大部分。化学连续提取法 (chemical sequential fractionation，CSF) 研究土壤磷形态分级，采用不同的化学提取剂，分级提取土壤中组成或分解能力接近的有机无机含磷化合物，是目前表征土壤磷素形态的重要方法。但该方法虽历经改进，仍难以确切反映土壤磷的实际组成，提取的不同磷形态间存在重叠，有机磷和无机磷组分分级存在一定的误差；不同分级磷组分对作物的有效性，需谨慎评估。核磁共振波谱技术 (nuclear magnetic resonance，NMR) 根据核磁共振波谱图上共振峰的位置、强度和精细结构来研究土壤中含磷化合物的分子结构。液相 ^{31}P-NMR 可以同时检测出土壤中多种磷组分，如正磷酸盐、磷酸单酯、磷酸二酯、膦酸脂、焦磷酸盐和多聚磷酸盐，识别土壤提取物磷形态，可将有机磷与无机磷分开。本文综述了应用 ^{31}P-NMR 技术研究土壤磷形态组分的一些进展，总结了样品制备过程、NMR 测试参数及在土壤磷形态转化研究中的应用。二维 ^{31}P-NMR 技术发展为鉴定分析土壤中更多种类的含磷化合物提供了契机。

关键词: 土壤磷形态；化学连续提取法；土壤磷组分；^{31}P-核磁共振波谱技术

Research advance in soil phosphorus fractionations and their characterization by chemical sequential methods and ^{31}P-NMR techniques

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Abstract: Transformation of soil phosphorus (P) is an important part in P cycle in ecological system, which influences soil P availability for crops. Soil total P includes organic and inorganic P. Chemical sequential fractionation (CSF) methods with different chemical extraction solvents are often used to determine different forms of P in soils. Despite the extensive use of these CSF procedures, there are obvious shortcomings, e.g. less accurately distinguishing inorganic and organic forms of P. Nuclear magnetic resonance (NMR) can be used to identify exactly molecular forms of P in soils. Some reports of ^{31}P-NMR technique to study soil P forms and transformation were reviewed in this paper, and advances in the preparation process of soil samples and ^{31}P-NMR analysis parameters were summarized. Two-dimensional ^{31}P-NMR spectroscopy will be developed to improve P fractionation in quantification in soils.

Key words: soil phosphorus forms; chemical sequential fractionation; soil phosphorus fractions; ^{31}P nuclear magnetic resonance (^{31}P-NMR)

磷是生命体中多种生物大分子如 DNA、RNA、ATP、磷脂的组分元素，对能量贮存、迁移和转化过程具有重要作用[1]。自然界中磷主要存在于土壤和海洋两大库中，大气中含量较少[2–3]。土壤缺磷曾是

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作物生产上限制因素之一，但多年来，磷肥施用量持续增加，耕地土壤有效磷含量显著提高[4−5]。用于生产磷肥的磷矿资源不可再生，预测全球磷矿资源在 50～100 年内将面临耗竭[6−7]。土壤中磷移动性较差，易被吸附固定，作物当季磷肥利用率不高。研究土壤磷分级方法，有助于科学地认识、利用土壤磷，防止过量施用磷肥，减少磷矿资源浪费，降低土壤磷流失对环境构成的污染风险[8−9]。

1　土壤磷形态组分

土壤全磷 (P) 含量约 10～1000 g/kg，与土层、质地、发育、利用方式与强度等有关。土壤磷分为无机磷和有机磷两大部分，含量比一般在 0.1～3 之间[3, 10]。

土壤无机磷以正磷酸盐为主，焦磷酸盐、无机聚磷酸盐、偏磷酸盐等少量存在，又可分为矿物态、水溶态和吸附态三种形态[11−14]。土壤无机磷约有 99% 以矿物态存在，难被植物吸收利用；石灰性土壤中矿物态磷主要是羟基磷灰石或氟磷灰石，酸性土壤中以铁铝氧化物及氢氧化物结合态磷为主[15−18]。土壤溶液中 $H_2PO_4^-$ 和 HPO_4^{2-} 离子，占全磷 < 0.1%，在土体中主要通过扩散作用迁移，是植物吸收利用的有效形态[11, 19]。吸附态磷是指通过范德华力、化学键能等吸附在粘土矿物、有机物等固相表面的磷，以阴离子交换吸附和配位吸附 (专性吸附) 为主[11, 17]。

根据分子结构差异，土壤有机磷分为磷酸酯、膦酸盐、多聚磷酸酯、微生物量磷等，还包括吸附在有机物表面和与有机物形成配合物的磷酸盐[10, 20−24]。磷酸酯类有机磷较易分解，在土壤有机磷中占很大比例，包括磷酸单酯类和磷酸二酯类：磷酸单酯通过羟基酯化，与 C 链相连，形成磷酸酯 (C-O-P) 形式，如磷酸糖类、单核苷酸、肌醇六磷酸 (植酸)[10]；磷酸二酯以 C-O-P-O-C 形式桥接，如磷脂类、核酸、脂磷壁酸等，农田土壤中磷酸二酯含量通常低于 10%[10, 25-26]。膦酸盐含碳磷键 (C-P)，如 2-氨基乙基膦酸、抗生素磷霉素、农药草甘膦 (N-膦酰基甲基-甘氨酸) 等。膦酸盐比磷酸酯键更稳定，在寒冷、湿润或酸性环境下容易累积。烟酰胺腺嘌呤二核苷酸磷酸和三磷酸腺苷 (ATP) 具有磷酸单酯和膦酸盐结构，有学者归之为多聚磷酸酯[10, 24]。微生物量磷是土壤中所有活体微生物细胞内所含的磷，在农田中约占土壤全磷的 0.4%～2.5%，草地土壤中，可达全磷的 7.5%[27]。微生物量磷含核酸 (75%) 、酸溶解性磷酯类 (20%) 、磷脂 (5%)，是土壤有机磷中较为活跃的部分，是植物磷素营养的重要来源[28−29]。Meta 分析结果表明，全球土壤微生物量 C：N：P 约为 60：7：1[30]。土壤中有机磷成分复杂，受浸提、分析技术限制，仍有大量组分未被鉴别[31]。

2　土壤磷化学分级

土壤磷化学分级是指用化学连续提取法表征土壤磷素形态，即用不同的化学提取剂分级提取土壤中化学组成相近或分解矿化能力较接近的无机或有机磷化合物[12, 14, 32−34]。

Chang 和 Jackson[35]提出了酸性土壤无机磷分级方法，后经 Peterson 和 Corey[36]改进，该分级体系将土壤无机磷分为易溶态磷 (提取剂 1 mol/L NH_4Cl) 、铝磷酸盐 (0.5 mol/L NH_4F)、铁磷酸盐 (0.1 mol/L NaOH) 、钙磷酸盐 (0.5 mol/L H_2SO_4) 、闭蓄态磷 (0.3 mol/L 柠檬酸钠–0.5 g/L 连二硫酸钠–0.1 mol/L 氢氧化钠)。该法不能很好地区分石灰性土壤中不同形态的钙磷酸盐 Ca-P。蒋柏藩和顾益初[37]提出石灰性土壤无机磷分级方法，把 Chang-Jackson 方法中 Ca-P 按溶解度和有效性又分为 3 级，分别是磷酸二钙型 Ca_2-P (提取剂 0.25 mol/L $NaHCO_3$) 、磷酸八钙型 Ca_8-P (1 mol/L NH_4OAc) 和磷石灰型 Ca_{10}-P (0.5 mol/L H_2SO_4)。将铁磷酸盐 (Fe-P) 改为 0.1 mol/L NaOH-Na_2CO_3 提取，这一方法在我国石灰性土壤磷形态研究中广泛应用。这些土壤磷分级方法主要缺陷是分级较粗，未包括有机磷组分，难以了解土壤磷总体变化。

测定土壤有机磷总量主要采用差减法，利用高温灼烧土样，促使有机磷分解，用酸提取，提取磷量减去未灼烧土壤样品提取磷量即为有机磷总量[38]。灼烧法操作简单，是测定有机磷总量经典方法，缺点是高温灼烧过程中矿物态磷溶解度可能发生变化，部分有机磷挥发损失。Bowman 和 Cole[39]将土壤有机磷分为活性、中等活性、中稳性和稳定性四种形态有机磷，分别用 0.5 mol/L $NaHCO_3$、1.0 mol/L H_2SO_4 和 0.5 mol/L NaOH 按顺序浸提；NaOH 浸提液经调酸后，沉淀部分为高稳性有机磷 (胡敏酸态有机磷)，不为酸所沉淀部分是中稳性有机磷 (富啡酸态有机磷)。Ivanoff 等[40]增加了微生物量磷组分，将中等活性有机磷的提取剂改为 1 mol/L HCl。活性有机磷易矿化而为植物吸收，中等活性有机磷较易矿化，中稳性有机磷较难矿化，难被植物吸收利用，高稳性有机磷很难矿化，基本上不被植物所吸收[14, 20]。

Hedley 等[41]提出土壤磷分级方法，被国内外学者普遍采用[12, 14, 32−34]。该法将土壤磷分为 7 大类，部

分类别又分为有机态 (Po) 和无机态 (Pi)：1) 树脂交换态磷　阴离子交换树脂交换浸提出的磷，主要是与土壤溶液中的磷处于平衡状态的土壤胶体吸附的无机磷，可被作物吸收；2) $NaHCO_3$ 提取态磷　包括无机态和有机态两部分，对植物有效；3) 微生物量磷　主要是来自微生物体内磷溶解浸提，包括有机和无机两部分，在适宜条件下，微生物量磷可较快地矿化后为植物利用；4) NaOH 提取态磷　包括有机和无机两部分；5) 土壤团聚体内磷　土壤经超声波分散，再用 0.1 mol/L NaOH 提取的磷，包括有机和无机两部分，主要是指存在于土壤团聚体内表面上的磷；6) HCl 提取态磷　在石灰性土壤中主要提取的是磷灰石型磷，高度风化的酸性土壤中能提取出部分闭蓄态磷；7) 残渣态磷　指以上试剂不能提取的较稳定的磷。

Condron 和 Goh[42]在 Hedley 分级方法基础上进行了改动，省去了微生物量磷测定，即 0.1 mol/L NaOH 浸提后用 1.0 mol/L HCl 浸提，样品不经超声波分散，直接用 0.5 mol/L NaOH 提取，省去土壤团聚体内磷这一形态。Chen 等[43]在此基础上又进行了部分修订：1 mol/L NH_4Cl 代替树脂浸提；第二次 NaOH 浸提浓度改为 0.5 mol/L；残渣态磷改为用 HNO_3–$HClO_4$ 消煮。Tiessen 等[44]对 Hedley 分级法也进行了修正，共分为 6 个大类 9 个分级，将 Hedley 分级法中含量较低的微生物量磷和团聚体内磷省去，在 0.1 mol/L 稀盐酸浸提后再用浓盐酸浸提，以充分提取残留态中的部分有机磷。Hedley 磷素分级及其修订的方法，为了充分提取土壤中磷，浸提液需在 25000 × g 下超高速离心，同时利用 0.45 μm 滤膜过滤，提取过程费时，测试成本较高，这些限制了方法的应用。Guppy 等[45]对 Hedley 分级方法进行改进，省去了微生物量磷测定，在浸提剂中添加 4 mol/L NaCl 溶液提高离子强度，增加土壤胶体絮凝性，离心力只需 900 × g 即可，无需过滤便得上清液，操作简便，测试成本较低。该法还采用孔雀绿或钼蓝比色法测定无机磷，孔雀绿比色法灵敏度更高；各形态全磷消煮后，用电感耦合等离子体发射光谱法测定，Guppy 法中各形态磷回收率达到了 95%。

化学连续提取法是通过选择浸提剂对土壤中磷进行区分提取，但浸提剂缺乏专一性，浸提过程中可能出现腐殖质沉淀、有机磷水解以及沉淀与螯合反应，导致一些无机磷和有机磷组分在浸提过程中难以真正完全区分开。浸提液中磷浓度测定多用钼蓝比色法，该方法简单易行，但钼蓝比色法测得磷 (molybdate-reactive phosphorus，MRP) 仅是与钼酸盐反应的正磷酸盐，聚磷酸盐、焦磷酸盐不与钼酸盐反应，难以被检出，被归入钼酸盐非反应磷 (molybdate-unreactive phosphorus，MUP)，MRP 与 MUP 并不能和无机磷与有机磷一一等同[46]。因此化学浸提方法提取的不同磷形态间存在重叠，有机磷和无机磷组分分级存在一定的误差；不同分级磷组分对植物的有效性，需谨慎评估[10, 14]。

3　利用 ^{31}P-NMR 技术研究土壤磷形态组分

3.1　^{31}P-NMR 技术基本原理

NMR 技术基于磁性原子自旋共振现象，是根据谱图上共振峰位置、强度和精细结构研究样品分子结构[47–48]。原子核是带正电荷具有质量的粒子，能自旋的原子核具有循环电流，产生磁场，形成磁矩 (μ)。无外加磁场时，自旋核取向是任意的。当自旋核处于磁感应强度 B_0 的外磁场中，绕磁场运动，称为拉莫尔进动，角速度 $\omega_0 = 2\pi\nu_0 = \gamma B_0$，式中 ν_0 是进动频率，γ 为磁旋比。磁原子核自旋量子数 $I = 1/2$，$\gamma = 10.829 \times 10^7$ rad/T/s，$\mu = 1.9581$。在外磁场作用下自旋量子数 I 值 1/2 的核有两种取向，用自旋磁量子数 m 表示，$m = +1/2$ 和 $-1/2$，这两种状态间存在能量差；当接受一定频率电磁波辐射，辐射能量等于自旋核两种不同取向的能量差时，处于低能态自旋核跃迁到高能态，称为 NMR。因此 NMR 基本条件是：频率为 $\nu_{射}$ 射频照射自旋核，射频能量 $E_{射} = h\nu_{射} = \Delta E = \gamma h B_0/2\pi$，即 $\nu_{射}$ = 拉莫尔进动频率 $\nu_0 = \gamma B_0/2\pi$，检测电磁辐射被吸收的情况得到 NMR 波谱[47]。不同化合物中磷原子核的化学环境不同，核外电子绕核运动产生与外部磁场方向相反的感应磁场，对原子核产生一定的屏蔽作用，核实际处于磁场强度 $B_0(1-\sigma)$ 的状态，σ 为屏蔽常数，发生 NMR 时，拉莫尔进动频率 $\nu_0=\gamma B_0(1-\sigma)/2\pi$[47–48]。核外电子对核的屏蔽作用不同导致不同磷化合物共振频率有微小移动，称为化学位移 δ。通过核磁共振仪测出 δ，可对不同化学环境的原子核进行定性[47–48]；磷 NMR 谱强度与磷原子核的浓度呈正比，通过谱图上特征峰积分对磷化合物进行定量分析。实际操作时，磁场强度 B_0 难以准确测定，δ 值确定常以待测物中磷原子核相对于参考物 (如 85% H_3PO_4) 磷原子核的吸收频率表示，$\delta=[(\nu_{样}-\nu_{标样})/\nu_0] \times 10^6$，单位为 ppm[21–22]。^{31}P 是自然界中磷元素唯一的天然稳定性同位素，自然丰度为 100%，理论上讲，样品中所有磷形态均可被 NMR 检测，但是土壤异质性、磷含量相对较低、磷

易与顺磁性铁锰离子结合，导致土壤样品 ^{31}P-NMR 分析较复杂[10, 49]。

3.2　土壤 ^{31} P-NMR 技术参数

Newman 和 Tate[50]首次将^{31}P-NMR 技术应用于土壤提取液中磷表征。^{31}P-NMR 技术包括固相和液相 ^{31}P-NMR。固相 ^{31}P-NMR 测定的样品前处理只需干燥、研磨，无需浸提，但其分辨率和灵敏度较低，目前应用不普遍。液相 ^{31}P-NMR 可检测出多种磷化合物，有效区分土壤有机磷与无机磷化合物，因此液相 ^{31}P-NMR 技术应用较广泛，但土壤液相 ^{31}P-NMR 技术尚存在一些问题，如不适于分析微量样品，提取及分析过程可能出现磷化合物水解[10, 21–22, 51]。

应用液相 ^{31}P-NMR 分析土壤磷组分的前提与关键是要进行样品制备和磷化合物提取。样品制备包括样品前处理 (干燥、研磨)，选择合适的浸提条件 (提取剂种类、提取时间及温度、提取剂用量)，样品待测液处理[10, 21–22, 51]，NMR 测试参数设计包括脉冲角度、弛豫时间、采集时间、温度、是否氢去偶等。Cade-Menun 等[10, 21]综述了土壤样品 ^{31}P-NMR 技术原理与应用，系统总结了样品的制备过程, 包括样品前处理方法、提取时间、提取剂比例以及核磁共振测试参数 (表 1)。

样品前处理包括样品干燥、研磨。报道的样品干燥方式有烘干[52–53]、自然风干[54–56]、冷冻干燥[57–58]，也有直接利用新鲜土壤样品[59–62]，多数研究者使用自然风干土壤样品[10, 21, 54–56, 63]。样品自然风干可能会带来正磷酸盐和磷酸单酯含量增加，磷酸二酯含量降低，冷冻储存新鲜样品则更接近原样品。对我国 43 个湖泊表层沉积物进行研究，发现风干样品较新鲜样品磷的提取率更高，样品风干增加了有机磷的水解，高温下风干会低估有机磷的含量；风干样品经充分研磨可破坏沉积物结构，尤其是对含矿物质多的沉积物，促进了磷的释放[64]。在充入 N_2 条件下浸提新鲜土壤样品，可防止样品中原有磷形态被氧化[63]。

用于 NMR 分析的土壤样品浸提剂有：0.1 mol/L NaOH%–0.4 mol/L NaF[63, 65–66]、水[67]、水 + 0.4 mol/L NaOH[68]、0.5 mol/L $NaHCO_3$ 和 1.0 mol/L HCl 连续浸提后用 0.5 mol/L NaOH 浸提[69]、水 + 0.5 mol/L $NaHCO_3$、NaOH-EDTA 连续浸提[70]、HCl–NaOH– 阳离子交换树脂 Chelex 多步提取[62, 71]、0.25 mol/L NaOH–50 mmol/L EDTA 两步提取[72]。土壤中 Ca、Fe、Al、Mn 等与磷结合，NaOH 提取有机磷并不完全，选择阳离子交换树脂、连二亚硫酸钠、NaF、稀酸、EDTA 等与 NaOH 一起联用，去除或螯合金属离子，释放出磷，提高提取效率。阳离子交换树脂去除阳离子过程中可能带走多聚磷酸盐，连二亚硫酸钠 – 碳酸氢钠将土壤 Fe^{3+} 转化为可溶 Fe^{2+} 离子，NaF 螯合 Al，EDTA 对 Ca、Fe、Al、Mn 等具有螯合作用，对有机磷组分破坏小，降低多聚磷酸盐水解，对有机磷的提取率较高[73]。土壤 ^{31}P-NMR 研究中，0.25 mol/L NaOH–50 mmol/L EDTA 是常用提取剂[10, 21–22, 51]。

选用适当的浸提剂与土样比例，对土壤磷提取效果及检测灵敏度非常重要。相同情况下，浸提剂与土样比例越高，各组分磷的浓度越低，被检测出来的可能性越小[74]。Cade-Menun 和 Preston[75]选择浸提剂体积与土样质量比例 (水土比) 20∶1，这一比例被广泛应用[10]，但其所用土样是有机质较高 (C 含量 50%) 的森林淹水土壤，对于矿质含量高的土样，Cade-Menun 等[73, 76–77]选择水土比 10∶1。Doolette 等[61]报道，与水土比 20∶1 相比，水土比 10∶1 提高了总磷回收率和 NMR 信噪比，但研究结果并未显示有机磷和正磷酸盐回收率是否增加。Turner[78]利用热带土壤样品，增加浸提水土比，浸提液 MRP 含量提高，MUP 未增加。对于低磷土壤，McLaren 等[79]认为 NaOH-EDTA 浸提水土比 4∶1 的 NMR 信号灵敏度好于水土比 10∶1。

浸提振荡时间一般选择常温下振荡提取 16 h。但也有 8 h[80]和 4 h[81–83]的报道。利用 NaOH-EDTA 浸提剂对热带森林土样分别浸提 1、4 和 16 h，4 h 浸提的总 P 和 MUP 量比 1 h 稍高，16 h 浸提总磷和 MRP 比 4 h 高，MUP 量并未增加，表明 16 h 浸提无机磷增多，有机磷并未增加[78]。浸提时间短可能会减少提取液中磷组分的水解和降解。除提取时间外，温度、pH 等因素也一定程度上影响提取液中磷组分及 NMR 的检测结果。温度升高增加有机磷矿化率及无机磷释放；氧气充足情况下磷酸二酯易降解。采用 0.2 mol/L 草酸铵 (pH 3.0) 按水土比 40∶1 振荡样品 2 h，在 2000× g 下离心 10 min，NMR 可检测到温带草地和森林土壤中肌醇六磷酸[84]。

提取完成后，浸提液需要进行浓缩以提高 NMR 样品管中磷浓度。若直接采用浸提样品，每个样品测试时扫描 112000 次，采集时间 0.4 s，弛豫时间 2.1 s，每采集信号一次约需 2.5 s，这样一个样品的 ^{31}P-NMR 分析时间约需要 78 h，无疑难以被接受[10]。以前研究者多采用冷冻干燥、40℃ 下氮吹、旋转蒸发等浓缩措施[21–22]，目前多采用离心和冷冻干燥浓缩

表 1 液相 ^{31}P-NMR 土壤磷研究的核磁测试参数

Table 1 Sample experiments parameters of solution phosphorus-31 nuclear magnetic resonance spectroscopy for soil analysis

土壤类型/利用方式 Soil type/Land use	溶解溶剂 Solvent	离心/过滤 Cent./Filt.	核磁管 (mm) Probe size	脉冲角度 Angle	采集时间 (s) Acquire time	弛豫时间(s) Delay time	温度 (℃) Temperature	扫描次数 No. of scan
森林 Forest[106]	D_2O	-	5	90	0.7	3.3	n.i.	5600
牧场 Grassland soil[107]	D_2O	-	10	90	0.1	0.2	27	n.i.
牧场 Grassland soil[52]	NaOH, D_2O	-	5	90	0.65	1	n.i.	240000
农田/牧场 Farmland/Meadow[108]	H_2O, D_2O	n.i.	10	90	1	10～19	24	17000
农田 Farmland[56]	NaOH-EDTA, NaOH, D_2O	+	5	90	0.68	4.32	20	5800
湿地/牧场 Wetland soil/ Meadow[60]	NaOH-EDTA, D_2O	-	10	30	0.4	1	n.i.	60000
湿地 Wetland soil[82]	NaOH-EDTA, D_2O	-	5	30	n.i.	2	25	30000
牧场/农田 Grassland soil/Farmland[93]	H_2O, D_2O	+	10	90	1	10～30	24	49000
农田 Farmland[61]	H_2O, D_2O	+	10	90	1	4	24	6858
湿地 Wetland soil[86]	D_2O	-	10	45	0.4	1.5	n.i.	n.i.
湿地 Wetland soil [102]	NaOH, D_2O	+	5	45	0.4	2	n.i.	n.i.
农田 Farmland[70]	D_2O	+	10	90	0.68	4.32	20	2900
牧场 Meadow[97]	NaOH, D_2O	-	10	90	0.82	1	n.i.	1024
农田/森林/湿地 Farmland/Forest/ Wetland soil[66]	NaOH, D_2O	n.i.	5	n.i.	n.i.	n.i.	17	n.i.
农田 Farmland[63]	NaOD	-	5	90	0.1	2	27	13000
农田 Farmland[109]	D_2O	-	5	45	0.8	5	20	14000
湿地 Wetland soil[110]	D_2O, NaOH	n.i.	5	45	1	2	n.i.	16384
牧场 Meadow[67]	H_2O, D_2O	+	10	45	1.99	1	20	18800
牧场 Meadow[89]	NaOH, D_2O	+	5	45	1.99	1	20	20000
原始土/牧场 Embryonic soil/Meadow[103]	D_2O	+	5	90	多样 Diverse	多样 Diverse	20	10000
牧场 Meadow[111]	NaOH, D_2O	+	10	45	1.99	5	20	9600
森林/农田 Forest/Farmland[95]	NaOH-EDTA, D_2O	-	5	45	0.4	0.4	n.i.	36000
农田/牧场 Farmland/Meadow[112]	NaOH, D_2O	n.i.	5	90	0.45	2	30	30000
农田 Farmland[62]	n.i.	n.i.	5	70	0.506	1	n.i.	15000
森林 Forest[65]	NaOD	-	5	90	0.1	0.2	17	n.i.
农田/肥料 Farmland/Fertilizer[68]	NaOD, D_2O	-	10	90	n.i.	2.5	2.5	15000
农田 Farmland[90]	H_2O, D_2O	+	10	90	1	15～20	24	n.i.
牧场 Meadow[54]	D_2O	+	10	90	0.68	4.32	20	2000
牧场/农田 Meadow/Farmland[56]	NaOH-EDTA, H_2O, D_2O	+	10	90	1	1	20	1200
湿地 Wetland soil[59]	D_2O	+	10	45	n.i.	2.1	n.i.	112000

续表 1　Table 1 continued

土壤类型/利用方式 Soil type/Land use	溶解溶剂 Solvent	离心/过滤 Cent./Filt.	核磁管 (mm) Probe size	脉冲角度 Angle	采集时间 (s) Acquire time	弛豫时间(s) Delay time	温度 (℃) Temperature	扫描次数 No. of scan
森林 Forest[83]	NaOH-EDTA, D_2O	-	5	45	0.4	2	n.i.	30000
湿地 Wetland soil[69]	D_2O	-	5	45	0.4	2	20	38000
原始土 Pedogenesis[94]	NaOH-EDTA, D_2O	-	5	45	0.4	2	n.i.	30000
森林 Forest[87]	D_2O	-	5	90	0.96	2	n.i.	3800
原始土 Pedogenesis[113]	D_2O, NaOD	+	5	45	0.9	2	n.i.	15000
湿地 Wetland soil[114]	n.i.	-	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
农田/森林 Farmland/Forest[101]	NaOH, D_2O	-	5	45	1.077	1	n.i.	26000
河岸带土 Riparian soils[115]	NaOH-EDTA, NaOH, D_2O	+	10	90	0.56	4.44	20	2900

注（Note）：表 1 参照文献[10]改编 Table 1 based on the reference[10]；n.i—文献中未提供任何信息 No information provided in the published study；“+” 代表 “是” Means yes、“-” 代表否 No；Cent./Filt.—样品加入核磁管前进行离心或者过滤 Centrifuging or filtering the prepared sample before putting it into the NMR tube；Temperature—核磁仪测定时样品室温度 Temperature in the sample cell when nuclear magnetic spectrometer is running.

浸提液为粉末样品[10, 74]。张艾明等[57]研究发现，浸提液冷冻干燥过程中添加连二亚硫酸盐缓冲液 (0.11 mol/L $NaHCO_3$–0.11 mol/L $Na_2S_2O_4$) 降低了 ^{31}P-NMR 谱图化学位移偏移，提高了分辨率。Cade-Menun 等[85]报道 NaOH-EDTA 浸提液冷冻干燥后，含有的三聚磷酸盐降解为正磷酸盐和焦磷酸盐；若浸提液 pH 中和至 7.0，三聚磷酸盐就不会降解。事实上，土壤 ^{31}P-NMR 图谱上很少报道聚磷酸盐，常见焦磷酸盐谱峰。

样品制备完成后，冷冻储存直至分析。^{31}P-NMR 分析前，取出冷冻浓缩样品，重新溶解，变成液体样品，准备待测样品体积根据 NMR 核磁样品管体积而定，5 mm 核磁管进样体积约 0.5～1 mL，10 mm 核磁管进样体积 2～3 mL。磁场漂移导致信号峰变宽，实验对磁场稳定性的要求可以通过锁场实现，锁场目前常用氘信号作为参照信号，通过不间断测量参照信号并与标准频率进行比较，调节偏差反馈到磁体通过增加或减少辅助线圈电流来进行矫正。核磁管样品中加入氘水 (D_2O) 是土壤 ^{31}P-NMR 常用锁场方法[10]。

重新溶解冷冻浓缩样品的溶剂：D_2O[71, 86–88]、氘代氢氧化钠 (NaOD)[52, 63]或NaOD+D_2O[68]、纯水+D_2O[89–92]、D_2O+NaOH-EDTA[93–96]、D_2O+1mol/L NaOH[97–99]、D_2O+10mol/L NaOH[54, 67, 100–101]、D_2O+NaOH-EDTA+10 mol/L NaOH[55, 70, 80]，不同溶剂对 ^{31}P-NMR 分辨率和检测结果有明显影响[10]。Cade-Menun[21–22]认为最终样品溶解液 pH>12，才能保证获得分辨率良好的谱图。核磁管中样品液粘稠会影响谱峰分辨率，NaOH 或 NaOH-EDTA 重新溶解冷冻浓缩样品，pH 高，会使溶液存在沉淀颗粒物，导致峰型变宽[10]。有研究者重新溶解冷冻浓缩样品后，离心[56, 70–71, 99, 102]或过滤[96]，上清液加入适量 D_2O 锁定信号，移至核磁管中进行 NMR 检测。

计算 ^{31}P-NMR 图谱不同组分的峰面积与所有磷化合物总峰面积的比例，可获得该组分所占全磷的比例信息，在样品测试时加入内标，与内标信号比对，可得到磷化合物绝对含量。亚甲基二膦酸盐 (methylene diphosphonic acid，MDP) 是常用内标物，MDP 可在样品冷冻浓缩后重新溶解时，加到核磁管中[97, 103]，也可将 MDP 加到土壤样品中，与样品进行同样浸提过程[91–92, 104–105]。

3.3　土壤 ^{31}P-NMR 研究

土壤 ^{31}P-NMR 图谱中常见含磷化合物 (NaOH-EDTA 提取) 的化学位移在 25～–25 ppm 间 (图 1)，分别为膦酸盐 20 附近、正磷酸盐 5～7、磷酸单酯 3～6、磷酸二酯 2.5～–1、焦磷酸盐–4～–5、多聚磷酸 盐主链末端磷–4～–5、多聚磷酸盐–20 附近。

^{31}P-NMR 技术普遍用于土壤磷组分分析。Madagascan 稻田土壤 NaOH-EDTA 提取磷中，有机磷占 19%～44%，多为磷酸单酯，DNA 少量，不到一半样品中检测到肌醇六磷酸 (Inositol Hexakisphosphate，IHP)[53]。在智利老成土上试验发现[62]，与燕麦/小麦轮作相比，羽扇豆/小麦轮作下土壤酸性磷酸酶活性增强，磷酸单酯比例增加；而燕麦/小麦轮作下正磷酸盐含量增多。应用 ^{31}P-NMR 技术研究我国东北地

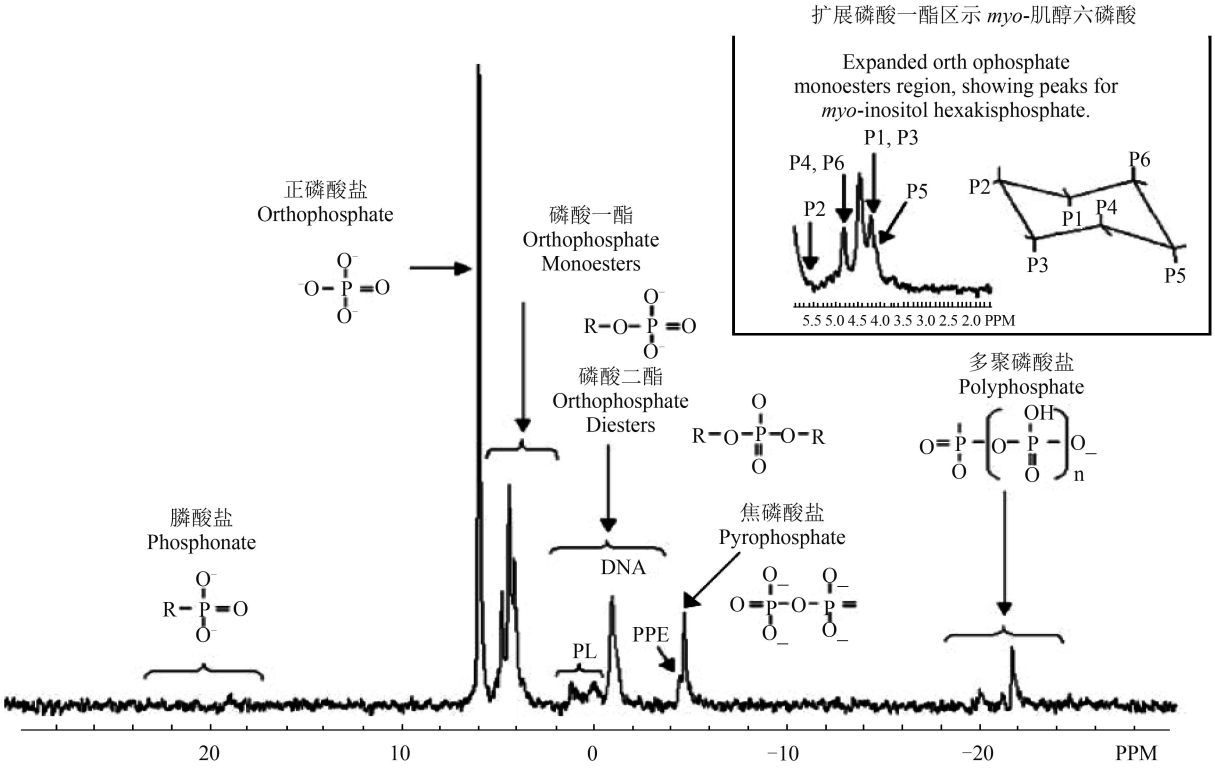

图 1 一个森林土壤 NaOH-EDTA 提取磷液相 ^{31}P-NMR 图谱[21, 98]

Fig. 1 Solution ^{31}P nuclear magnetic resonance spectrum of a forest floor sample extracted with NaOH-EDTA[21, 98]

区土壤，结果发现，棕壤和黑土中正磷酸盐和磷酸单酯分别约占总磷一半，褐土中主要磷组分为正磷酸盐，磷酸单酯占总磷 18%；棕壤和黑土中焦磷酸盐含量较高。棕壤和褐土鉴定出 *myo*-IHP，黑土检有 *scyllo*-IHP[116]。污泥施用增加了砖红壤和灰潮土土壤有机磷含量[117]。彭喜玲等[118]发现，NaOH-EDTA 浸提土壤磷占 NaOH 熔融法测定总磷的 54%～93%，污泥施用后 14 d，土壤正磷酸盐含量增加，磷酸单酯和焦磷酸盐含量下降。

应用 ^{31}P-NMR 技术研究土壤腐殖质中磷形态。腐殖酸结合态磷以磷酸单酯为主，磷酸二酯次之，有少量膦酸盐、正磷酸盐和焦磷酸盐；而富里酸结合态磷中磷酸二酯和正磷酸盐的比例较高[119]。寒冷潮湿气候条件下，高加索山地土壤腐殖酸中膦酸盐和磷酸二酯含量较高[120]。菲律宾水稻田土壤游离腐殖酸中磷酸二酯随水稻种植密度增加而累积，淹水种植三季水稻后，磷酸二酯含量占总磷比例达 42%，未淹稻田中占总磷 28%[121]。土壤游离腐殖酸和钙结合腐殖酸中活性无机磷占 10%；有机磷以磷酸单酯为主，磷酸二酯次之，膦酸盐少量 (< 3.7%)，检测到 *scyllo*-IHP，未检出焦磷酸盐或多聚磷酸盐[122]。

利用 ^{31}P-NMR 技术研究肥料施用对土壤磷组分影响。美国 6 个州 10 处土壤施用磷肥，正磷酸盐含量显著增加，磷酸单酯影响不明显[112]。施用有机肥 8 年以上的土壤与对照相比，IHP 含量未出现明显变化[98]。施用粪肥 11 年非钙质沙土剖面中，表层磷酸单酯累积，40～50 cm 土层正磷酸盐含量较表层高，土壤对磷酸单酯固持能力可能相对较强，正磷酸盐向下移动性相对较高[97]。粪肥中磷主要以无机态为主，鸡粪中 IHP 含量高于牛粪；潮土上施用粪肥，有机磷增加；随时间延长，磷酸单酯含量降低，核酸等磷酸二酯含量增加[123]。加拿大有机肥施用 20 年以上的长期牧场土壤有机磷尤其是磷酸二酯所占比例高于传统种植体系，其中磷酸二酯易矿化，可有效补充土壤磷供应，保证有机肥施用下土壤磷的有效供应[124]。向钙质土壤中添加 P 58 mg/kg IHP，十三周内迅速减少至初始添加量的 12%，伴随α及β甘油磷酸盐含量上升，表明微生物代谢作用导致 IHP 矿化，IHP 可作为钙质土壤中一种潜在有机磷源[61]。

利用 ^{31}P-NMR 技术与酶添加结合，深入研究有机磷组分分解矿化特性[70, 125–126]。牛粪、堆肥和干污泥中有机磷组分不同，瑞士微酸性的淋溶土上 62 年施用牛粪、堆肥和干污泥，表层土壤中 *myo*-IHP、*scyllo*-IHP、焦磷酸盐、磷脂类和核酸的降解产物等含量却无明显差异，可能与有机磷转化分解和淋失有关；在 NaOH-EDTA 浸提液添加磷酸酶、植酸

酶、核酸酶后，发现施用干污泥土壤中非水解磷积累[126]。

利用 ^{31}P-NMR 研究耕作对土壤磷形态的影响。加拿大魁北克玉米/大豆轮作长期定位试验结果表明，免耕小区深层土壤中磷酸单酯尤其是 *scyllo*-IHP 和核酸含量较高，可能与这些有机磷化合物从表层向底层迁移有关[127]。黑土和潮土上，免耕和秸秆还田增加了土壤磷酸单酯和磷酸二酯含量及在 NaOH-Na_2EDTA 浸提磷中的比例[128]。

分析土壤磷形态多采用一维 ^{31}P-NMR 技术，获得谱线有些过于拥挤、重叠，一些含磷化合物难以分辨[3, 51]。在实验中通过改变脉冲序列，加入另一段自由演化时间，引入 2 个时间变量，采集不同演化时间长度的信号，经过 2 次傅里叶变换后，得到两个独立的频率变量及耦合产生的交叉信息，产生具有两个独立时间变量二维 NMR 谱，可降低谱线拥挤和重叠程度。化学位移相关谱 (correlation spectroscopy，COSY) 是常用的二维NMR谱，X-Y 两个坐标轴都是化学位移信息，主要观测彼此间存在J耦合作用的原子核。Petzold 等[129]建立 ^{31}P-^{1}H COSY 谱，分析研究幽门螺杆菌细胞膜系统中磷脂组分，采用 Semiconstant-time 模式，设定 $2\Delta = 20$ ms，$t_{1,\max} = 60$ ms，减少了弛豫时间，提高了 ^{31}P 谱的信号分辨率，鉴定出磷脂酰乙醇胺、磷脂酰甘油、*sn*-2 溶血磷脂酰乙醇胺、磷脂酰甘油、卵磷脂、*sn*-2 溶血磷脂胆碱、*sn*-2 溶血磷脂酰乙醇胺血浆酶原、胆固醇基葡萄糖磷酸酯衍生物等。二维近程氢磷异核单量子相关谱 (Two-Dimensional 2D ^{31}P-^{1}H HSQC，Heteronuclear Single Quantum Correlation) 提供与磷核相耦合的烷基或烷基酯基团结构 P-O-CH_n 信息，为土壤磷化合物鉴定分析提供了技术基础。

Vestergren 等[88]利用 2D ^{31}P-^{1}H HSQC 技术鉴定出的北温带北部森林地区腐殖土有机磷化合物数量和种类明显比一维液相 ^{31}P-NMR 多。土壤浸提液冷冻干燥后，将样品溶解于 D_2O 中，添加 Na_2S 溶液，室温下放置 18～20 h，7000 *g* 下离心 30 min，沉淀去除 Fe 等顺磁物质干扰。经过 Na_2S 处理土壤浸提液 1D ^{31}P-NMR 图谱分辨率明显好于未经 Na_2S 处理的 1D ^{31}P-NMR 图谱，可将在一维谱图上与 *scyllo*-IHP和 α-磷酸甘油谱线重叠的化合物分开。Vincent 等[105]利用 2D ^{31}P-^{1}H NMR 技术分析瑞典北温带北部森林地区具有 7800 年历史的腐殖土层中磷，发现年轻土层中 α-磷酸甘油、β-磷酸甘油、核酸、焦磷酸盐含量较高；DNA、2-氨基乙基膦酸、多聚磷酸盐含量在 1200～2700 年历史的土层中明显较高；IHP 含量在不同年龄土层中波动变化。

土壤磷形态研究传统方法常用化学连续浸提法，但浸提剂缺乏专一性，不同磷分级之间存在相互干扰等，难以确切反映土壤磷的真正组分，尤其对有机磷化合物种类无法区分。^{31}P-NMR 技术可用于表征土壤中磷化合物，极大地促进了土壤磷形态及转化机制研究[3, 10, 92]，但在土壤磷提取、NMR 制样和测定过程，如何保证化合物不被降解，保持土壤中磷组分的原状信息，同时又有效提高 NMR 对磷化合物的分辨能力，是今后 ^{31}P-NMR 技术的重要研究内容。2D ^{31}P-^{1}H NMR 技术为鉴定分析土壤中更多种类的有机磷化合物提供了契机。

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