Localized application of soil organic matter shifts distribution of cluster roots of white lupin in the soil profile due to localized release of phosphorus

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INTRODUCTION

Cluster roots, also known as proteoid roots, are defined as clusters of hairy and determinate rootlets on a lateral root (Purnell, 1960). Cluster roots have been reported in a wide range of families, in species occurring on soils with a low availability of phosphorus (P), particularly in Australia and South Africa (Dinkelaker et al., 1995; Shane and Lambers, 2005). Cluster roots are also found in some Fabaceae, including *Lupinus albus* L. (white lupin). *Lupinus albus* is an important grain and forage crop widely cultivated around the Mediterranean and along the Nile valley (Huyghe, 1997), as well as in Australia (Gladstones, 1994). Like other cluster-root-forming species, under P deficiency, *L. albus* increases cluster-root formation, proton release and exudation of citrate (Gardner et al., 1983; Watt and Evans, 1999; Shen et al., 2005; Wang et al., 2007). Cluster roots have a large root surface to soil volume ratio, which enhances uptake of poorly mobile nutrients from soil (Vance et al., 2003; Lambers et al., 2006). More importantly, rapid exudation of protons, carboxylates and acid phosphates effectively mobilizes P and micronutrients (Dinkelaker et al., 1989; Wasaki et al., 2003). *Lupinus albus* is considered a model plant for cluster-root formation (Shen et al., 2003; Lambers et al., 2006).

Plant roots can respond to heterogeneous soil environments by increased initiation and growth of lateral roots (Drew, 1975; Gersani and Sachs, 1992; Van Vuuren et al., 1996). Cluster roots in species of Australian and South African Proteaceae tend to be concentrated in soil just below the litter layer (Jeffrey, 1967; Lamont, 1973). In an experiment with stratified soils and two *Hakea* species, there was a stronger formation of cluster roots in the humus-rich *A0* soil layer in comparison with that in other soil layers, independent of its location in the soil profile (Lamont, 1973). This stimulation of cluster-root formation by organic matter (OM) might be due to the release of nutrients from the OM. However, roots might also sense and respond to changes in soil strength associated with the presence of OM (Barley, 1962). Moreover, some phytohormones or other growth-promoting substances produced by micro-organisms involved in the decomposition of OM (Pizzeghrello et al., 2002) might also play a role in cluster-root formation, e.g. auxin stimulates cluster-root formation (Gilbert et al., 2000; Skene and James, 2000).

In a previous study, after inoculation with a rhizosphere soil extract of cluster roots, 4-month-old plants of Banksia grandis grown in γ-irradiated soil formed cluster roots, whereas the control plants did not; shoots of inoculated plants were twice...
as large as those of the non-inoculated plants (Malajczuk and Bowen, 1974). This led the authors to suggest that proteoid roots are microbially induced. However, it is equally possible that P released during the breakdown of OM was responsible for cluster-root induction. Moreover, cluster roots in *L. albus* are formed under axenic conditions (Gardner et al., 1982), showing that micro-organisms are not essential for cluster-root formation. However, compared with the axenic conditions, many more cluster roots are produced when micro-organisms are present (Gardner et al., 1982). The response of cluster-root formation to micro-organisms may be indirect, and due to the release of nutrients from OM.

P is one of the most important nutrients affecting cluster-root formation (Dinkelaker et al., 1995; Neumann et al., 1999; Shane and Lambers, 2005). Formation of cluster roots in severely P-impoverished soils is enhanced by a slight increase in P supply (Reddell et al., 1997; Shane et al., 2004), but strongly suppressed by a high P supply (Marschner et al., 1987; Moraghan, 1991). However, the nature of the response of cluster-root formation to OM is still poorly understood. The objectives of this study were to investigate the effect of localized application of OM on formation of cluster roots of *L. albus* grown in a P-deficient soil through a stratified soil column system, in which the soil profile was stratified into three layers, and either OM, phytate supplied in the presence of OM, or perlite was applied to the top or middle layers with or without sterilization of the soil and OM. This approach was chosen to test if the stimulating effect of OM on cluster-root formation was due to (a) P released from breakdown of OM; (b) a decrease in soil density; or (c) effects of micro-organisms other than releasing P from OM.

**MATERIALS AND METHODS**

**Soil preparation**

A soil with a low concentration of available P was collected from the foot of Tianzhu Mountain in Taian City in the Shandong province of China. Any discernible root pieces were removed from the soil. The air-dried soil was sieved (2 mm) and thoroughly mixed with washed river sand at a ratio of 1 : 1 (w/w) to create a P-deficient substrate (Table 1).

The P-sufficient substrate was established by adding KH₂PO₄ at 330 mg P kg⁻¹ soil. P-deficient substrate was mixed at a ratio of 1 : 1 (v/v) with peat (as OM) or perlite (perlite mix). The perlite mix gave a substrate with similar density to that of the soil mixed with OM. The phytate (sodium salt, 20 mg P kg⁻¹ soil) was applied in the presence of OM to test the role of P released from organic P in cluster-root formation. Soil and mixtures were placed in sterilization bags and sterilized by autoclaving at 120 °C for 2 h to be used in plastic tubes. Their major properties are listed in Table 1. Nutrients, except P, were added to all treatments and mixed at the following rates (mg kg⁻¹ soil): K₂SO₄, 120; Ca(NO₃)₂, 170; ZnSO₄·7H₂O, 7.5; CuSO₄·5H₂O, 5.3; Na₂MoO₄·5H₂O, 0.27; MgSO₄·4H₂O, 10.8; Fe-EDTA, 36.2.

**Plant cultivation**

The experiment was conducted in a greenhouse with natural light, with day/night temperatures of 22/10 °C. Seeds of white lupin (*L. albus* L. cv. Kiev) were germinated and seedlings were grown in plastic tubes (diameter × height = 100 × 400 mm) containing 5 kg of air-dried soil. After germination, plants were thinned to two per pot. The soil profile was stratified into three layers (0–10, 11–20 and 21–35 cm). There were nine treatments (Table 2): T1, homogeneously P-deficient substrate in the soil profile; T2, OM in the top layer and P-deficient substrate layer in the middle and bottom layers; T3, OM in the middle layer and P-deficient substrate in the top and bottom layers; T4, OM and P (330 mg P kg⁻¹ soil as KH₂PO₄, a P-sufficient treatment) in the top layer and P-sufficient substrate in the middle and bottom layers; T5, OM and P (as above) in the middle layer and P-sufficient substrate in the top and bottom layers; T6, phytate (sodium salt, 20 mg P kg⁻¹ soil) mixed with OM in the middle layer and P-deficient substrate in the top and bottom layers to test the role of P released from organic P in OM on cluster-root formation; T7, sterilized OM in the middle layer and sterilized P-deficient substrate in the top and bottom layers; T8, OM in the middle layer and sterilized P-deficient substrate in the top and bottom layers; T9, perlite mix in the middle layer and P-deficient substrate in the top and bottom layers.

There were two holes at the bottom of each pot for irrigation by capillary action. Water entered into pots through these holes to minimize nutrient movement between different layers caused by watering (He et al., 2003). There were four replicates of each treatment.

**Plant harvest**

After 60 d growth, shoots were severed at the soil surface. Roots were recovered from each layer separately and washed with deionized water. Cluster roots were defined as those secondary laterals with ≥10 rootlets cm⁻¹ (Johnson et al., 1994). All roots in each layer were scanned (Epson Expression 1600 pro, Model EU-35, Japan). Root length was calculated by the WinRHIZO image analysing system (WinRHIZO Pro2004b, version 5.0, Canada) from scanned root images. Shoots and roots were dried at 70 °C for 3 d and weighed.

**Sampling of root exudates and measurement of carboxylates**

At harvest, three cluster roots (if there were <3 cluster roots in a layer, all of them were taken) in the middle layer were cut and...
The mobile phase was 25 mM KH$_2$PO$_4$ (Cawthray, 2003). Separation was conducted on a 250 mm, 4.6 mm reversed phase HPLC in the ion suppression mode (Alltima C-18, Alltech, USA) using the vanado-molybdate method (Westerman, 1990). The sub-samples of root exudates were kept at –18 °C and gently shaken to remove the activity of micro-organisms. The solution was filtered, and total P was assayed using the vanado-molybdate method (Westerman, 1990).

Cluster roots were transferred to a tube of suitable size containing 10 mL of ultrapure water and gently shaken to remove the rhizosphere soil. This soil extract is referred to as the ‘root exudates’. Afterwards, Micropur (Sicheres Trinkwasser, Germany) was added to the root exudates to inhibit the activity of micro-organisms. The sub-samples of root exudates were kept at –18 °C until analysis. Rhizosphere soil in tubes was oven dried and weighed.

The organic acid anions in root exudates were analysed by reversed phase HPLC in the ion suppression mode (Cawthray, 2003). Separation was conducted on a 250 × 4.6 mm reversed phase column (Alltima C-18, Alltech, Deerfield, IL, USA). The mobile phase was 25 mM KH$_2$PO$_4$ (pH 2.5), with a flow rate of 1 mL min$^{-1}$ at 28 °C and UV detection at 214 nm. The sample injection volume was 20 μL. Identification of organic acids was performed by comparing retention times and absorption spectra with those of known standards.

**Measurement of P concentration of plant tissues**

The ground plant material was ashed at 580 °C for 10 h, and then dissolved in 2 mL of 1 : 1 (v/v) HNO$_3$ and 18 mL of deionized water. The solution was filtered, and total P was assayed using the vanado-molybdate method (Westerman, 1990).

**Statistics**

Analysis of variance was conducted using the SAS statistical software (SAS, 2001).

**RESULTS**

**Plant growth**

Dry mass of shoots, roots and whole plants in the treatments with P addition was greater than that in all other treatments without P addition (Fig. 1). Dry mass of whole plants in treatments T4 and T5 with P addition increased by 30 and 50 %, respectively, in comparison with T1 with P-deficient substrate. There was no difference in dry mass of whole plants among treatments without P addition. Interestingly, for the treatments with P addition, shoot dry mass in treatment T5, with OM in the middle layer, was 19.2 % greater than that in treatment T4, with OM in the top layer. However, no difference was found in root dry mass between T4 and T5.

When OM was located in the top layer of the soil profile in treatments T2 and T4, there was 199 and 88 % more root dry mass, respectively, in the top layer than in the middle layer (Table 3). In contrast, localized application of OM in the middle layers, in the treatments T3 and T5, did not increase root dry mass in the middle layer, in comparison with placement of OM in the top layer, regardless of P supply. Application of phytate plus OM in the middle layer of treatment T6 (to test the possible effect of P mobilization from organic P sources) increased the root dry biomass by 30 % in the middle layer compared with that in the corresponding OM layer in treatment T3. Similarly, non-sterile OM application in T8 (OM in the middle layer and sterile top and bottom layers) significantly increased root dry biomass in the middle layer compared with that in the sterile OM layer in treatment T7 (sterilized OM in the middle layer and sterilized P-deficient substrate in the top and bottom layers). Both the sterile OM (T7) and perlite application (T9) in the middle layers caused a significant decrease in root biomass in the middle layer in comparison with that in the top layer. Total root dry biomass of plants in treatments with P addition was significantly greater than that in the other treatments without P addition.

In treatment T1 with a P-deficient substrate in the whole soil profile, root length was greater in the middle layer than in the

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**TABLE 2. Vertical sections of different treatments**

<table>
<thead>
<tr>
<th>Layer (cm)</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>T6</th>
<th>T7</th>
<th>T8</th>
<th>T9</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–10</td>
<td>P-deficient</td>
<td>OM</td>
<td>P-deficient</td>
<td>OM</td>
<td>P-sufficient</td>
<td>P-deficient</td>
<td>Sterilized</td>
<td>P-deficient</td>
<td>P-deficient</td>
</tr>
<tr>
<td>11–20</td>
<td>P-deficient</td>
<td>P-deficient</td>
<td>OM</td>
<td>P-deficient</td>
<td>P-sufficient</td>
<td>OM</td>
<td>Phytate + OM</td>
<td>Sterilized OM</td>
<td>OM</td>
</tr>
<tr>
<td>21–35</td>
<td>P-deficient</td>
<td>P-deficient</td>
<td>OM</td>
<td>P-deficient</td>
<td>P-sufficient</td>
<td>P-deficient</td>
<td>Sterilized</td>
<td>P-deficient</td>
<td>P-deficient</td>
</tr>
</tbody>
</table>

P-deficient, P-deficient substrate; P-sufficient, P-sufficient substrate; OM, organic matter mixed with soil; Phytate + OM, phytate mixed with OM and soil; Sterilized OM, sterilized OM mixed with soil; Sterilized P-deficient, sterilized P-deficient substrate; Perlite mix, perlite mixed with P-deficient soil

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**FIG. 1.** Dry biomass of shoots and roots of *L. albus*. For explanations of the treatments, see Table 2. Different letters denote significant differences ($P < 0.05$) within the components of dry mass: letters above the columns indicate the whole plant, and roots and shoots are indicated alongside the shaded and unshaded sections, respectively.
Enhanced cluster-root dry biomass. Total dry mass of cluster-root OM (T6) and non-sterile OM application (T8) significantly increased in the middle layer compared with T3. Phytate application in the presence of OM or OM plus phytate, and non-sterile OM applied in the sterile soil profile with P-deficient substrate (Table 4). In contrast, sterilization of OM (T7) decreased dry biomass and number of cluster roots. The application of perlite in the middle layer (T9), to test the effect of a decreased soil density, also decreased the number of cluster roots compared with that in the corresponding treatments (T3, T1).

In treatment T1, with a homogeneously low P supply throughout the soil profile, the proportion of cluster roots to total roots in dry weight in each layer varied with depth; the proportion of cluster roots in the top layer was lower than that in the other layers. Localized application of OM (T3, T8) or phytate plus OM (T6) in the middle layers increased the proportion of cluster roots in comparison with that in the corresponding top layers. However, OM in the top layer (T2) increased the proportion of cluster root in the top layer and decreased it in the middle layer in comparison with treatment T1, with a homogeneously low P supply throughout the soil profile. When P supply was sufficient, the stimulating effect of localized application of OM became weak in treatments T4 and T5. Sterilization of OM (T7) significantly decreased the proportion of cluster roots to total root biomass in comparison with the treatment of non-sterilized OM (T8). In combination with the effects of treatment T6 where phytate was used in combination with OM, this indicates a role for microorganisms in cluster-root formation through release of P from OM. The proportion of cluster roots to total root biomass was lower in treatments with P addition, T4 and T5, than that in the other treatments without P addition. There was no significant difference in the proportion of total cluster roots to total root biomass among treatments with P addition.

In treatment T1, the dry weight of cluster roots as a percentage of that of total cluster roots of plants was 8% in the top layer and 46% in the middle layer (Fig. 2). The percentage of cluster roots increased to 53 and 37% in the top layer in treatments T2 and T4, respectively, with OM application in the top layer. In treatments T3, T5, T6 and T8, most of the cluster roots were concentrated in the middle layer where...
OM or phytate was located, with the proportion of cluster roots being 93, 95, 97 and 89 %, respectively. Interestingly, in treatment T7 with sterile OM in the middle layer and no opportunity to release P microbially, the cluster-root dry weight in that layer as a percentage of total cluster-root dry weight was decreased to only 20 %.

Citrate in the rhizosphere

The citrate concentration in the rhizosphere of cluster roots varied greatly in the middle layer, from 1.4 to 11.4 μmol g⁻¹ dry soil (Fig. 4). The lowest rhizosphere citrate concentration with cluster roots in the OM layer was observed in treatment T4 and T5 (with P addition) and the highest in treatment T3 (without P addition). There was no significant difference in citrate concentration among the treatments with P-deficient substrate, but phytate plus OM application in the middle layer of treatment T6 (with P-deficient substrate) decreased the citrate concentration in the rhizosphere soil of cluster roots. No or little citrate was detected in the bulk soil (data not shown).

P concentration in plant tissues

A significant increase in shoot P concentration was observed in treatments T4 and T5, with P addition, compared with the treatments without P addition (Fig. 5A). For treatments with P addition, shoot P concentration of plants in treatment T4 (OM in the top layer) was higher than that in treatment T5 (OM in the middle layer). There was no significant difference in shoot P concentration among treatments without P addition, except for the partly sterilized treatment T8, which had a relatively high shoot P concentration. Root P concentration was similar in all the treatments without P addition (Fig. 5B). Root P concentration was higher in treatments with P addition compared with treatments without P addition. Root P concentration varied strongly in different layers of treatments with P addition, T4 and T5. Root P concentration and P content in the middle layer increased by 224 and 109 % (calculated from Table 3 and Fig. 5) in treatment T4 (OM in the top layer) compared with that in treatment T5 (OM in the middle layer). There was no significant difference in P content in shoots or roots among the treatments without P addition (calculated from data in Table 3 and Fig. 5, data not shown).

DISCUSSION

Plant root biomass and root length

In the present study, localized application of OM significantly increased root length of L. albus in the layer where OM was applied, except for treatment T7, where the soil was sterilized. Root dry biomass was significantly increased when OM was applied to the top layer, but not when OM was applied to the middle layer. However, localized application of OM in

Table 4. Dry biomass and number of cluster roots and proportion of cluster roots to total roots (dry mass) of L. albus in different soil layers

<table>
<thead>
<tr>
<th>Root analysis</th>
<th>Horizon</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>T6</th>
<th>T7</th>
<th>T8</th>
<th>T9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cluster-root dry biomass (mg column⁻¹)</td>
<td>0–10</td>
<td>2.9</td>
<td>18.1</td>
<td>0</td>
<td>23</td>
<td>0.9</td>
<td>1.6</td>
<td>13.4</td>
<td>1.3</td>
<td>12.9</td>
</tr>
<tr>
<td></td>
<td>11–20</td>
<td>2.9</td>
<td>14.2</td>
<td>1.4</td>
<td>13.6</td>
<td>31.4</td>
<td>8.9</td>
<td>34.2</td>
<td>23.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>21–35</td>
<td>21.7</td>
<td>7.5</td>
<td>0.3</td>
<td>0.2</td>
<td>19.3</td>
<td>2.4</td>
<td>4.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD₀.₀₅</td>
<td></td>
<td>17.5</td>
<td>14.9</td>
<td>11.3</td>
<td>3.8</td>
<td>5.5</td>
<td>10.4</td>
<td>11.9</td>
<td>13.1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>46.5</td>
<td>37.0</td>
<td>18.7</td>
<td>9.3</td>
<td>17.8</td>
<td>33.0</td>
<td>41.6</td>
<td>37.9</td>
<td>40.85</td>
</tr>
<tr>
<td>LSD₀.₀₅</td>
<td></td>
<td>19.2</td>
<td>7.0</td>
<td>4.4</td>
<td>2.1</td>
<td>4.0</td>
<td>6.0</td>
<td>2.1</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>No. of cluster roots per column</td>
<td>0–10</td>
<td>2.6</td>
<td>17.0</td>
<td>0</td>
<td>3.6</td>
<td>1.0</td>
<td>0.1</td>
<td>11.1</td>
<td>1.9</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>11–20</td>
<td>11.1</td>
<td>25.4</td>
<td>14.3</td>
<td>4.1</td>
<td>13.6</td>
<td>20.4</td>
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</tr>
<tr>
<td></td>
<td>21–35</td>
<td>5.8</td>
<td>4.0</td>
<td>0.3</td>
<td>1.4</td>
<td>0.4</td>
<td>1.4</td>
<td>6.0</td>
<td>2.1</td>
<td>0.8</td>
</tr>
<tr>
<td>LSD₀.₀₅</td>
<td></td>
<td>4.6</td>
<td>5.9</td>
<td>6.9</td>
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<td>9.9</td>
<td>7.0</td>
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<tr>
<td>Total</td>
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<td>19.5</td>
<td>23.5</td>
<td>14.6</td>
<td>9.1</td>
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<td>26.0</td>
<td>30.9</td>
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<tr>
<td>LSD₀.₀₅</td>
<td></td>
<td>9.8</td>
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<td>9.8</td>
<td>9.8</td>
<td>9.8</td>
<td>9.8</td>
<td>9.8</td>
</tr>
</tbody>
</table>

For explanations of the treatments, see Table 2.

Fig. 2. The percentage of dry mass of cluster roots in each layer in relation to total dry mass of cluster roots of L. albus. For explanations of treatments, see Table 2. Different letters for each column denote significant differences between layers within that treatment (P < 0.05).

OM or phytate was located, with the proportion of cluster roots being 93, 95, 97 and 89 %, respectively. Interestingly, in treatment T7 with sterile OM in the middle layer and no opportunity to release P microbially, the cluster-root dry weight in that layer as a percentage of total cluster-root dry weight was decreased to only 20 %.

Citrate in the rhizosphere

For explanations of the treatments, see Table 2.
the middle layer stimulated fine root proliferation more than application in the top layer (Table 3, Fig. 3), leading to a greater specific root length (calculated from Table 3, data not shown). Increased growth of lateral roots has been demonstrated in barley (*Hordeum vulgare* L.) in response to localized supply of inorganic P and/or N (Drew, 1975; Drew and Saker, 1978; Weligama et al., 2008). In the present study, it is unlikely that the response of root length to OM was caused by N supply, because we added adequate nitrate to all layers so as to minimize the difference in N content between the substrates. Original Olsen P in OM was around twice as great as in soil layers without additional P. Greater root length in the layer with OM was probably partly due to higher P availability in the OM layer in P-deficient treatments. This positive effect of OM on root length was also observed in P-sufficient treatments of T4 and T5, but became weak with increased P supply in the soil substrate.

Localized application of phytate mixed with OM in the middle layer (T6) significantly enhanced both root dry biomass and root length in the middle layer compared with that in the corresponding layer of treatment T3 with only OM addition, suggesting a clear effect of P mobilized from organic P by micro-organisms. Non-sterile OM application in the middle layer in treatment T8 (OM in the middle layer and sterilized P-deficient substrate in the top and bottom layers) also increased both root dry biomass and root length. Micro-organisms can stimulate root growth through releasing auxin (Barazani and Friedman, 1999) or mobilizing nutrients (Gyaneshwar et al., 2002). When soil was sterilized, stimulation of root growth by OM was absent, indicating that micro-organisms involved in the breakdown of OM play a role in stimulation of root growth. Soil nutrient availability is expected to also be enhanced by autoclaving; this might also enhance root growth (Alef, 1995).

In a previous study, an increase of root biomass and length was observed in patches of soil with low density for barley (*H. vulgare* L.) and wheat (*Triticum aestivum* L.) (Bingham and Bengough, 2003). However, in the present study, root dry biomass and length of *L. albus* were not stimulated by low density when perlite was added, indicating that the lower density did not account for the greater root density in the OM layer.

**Formation and distribution of cluster roots**

Localized application of OM induced proliferation of cluster roots, regardless of the position of the OM layer, though there was a higher frequency of cluster-root formation in the middle layer compared with that in the top layer, i.e. in T1, with a homogeneously low P supply throughout the soil profile. However, this stimulating effect of OM on cluster-root proportion became weak when sufficient P was applied to the whole soil profile in treatments T4 and T5. The results suggest that the response of cluster-root proliferation to localized application of OM is highly dependent on P status of the plants. Similarly,
cluster roots of *Hakea laurina* were stimulated by OM at any depth within the soil profile (Lamont, 1973). The maximal proportion of cluster roots to total root dry biomass was 38% in Lamont’s experiment (calculated from Lamont, 1973). This is in agreement with the results of the present study with *L. albus*, where the proportion of cluster roots to total root dry biomass was 34–44% in the middle layer with OM, but only 0–3% in the rest of soil profile in most P-deficient treatments. This indicates that cluster roots were predominantly located in the layers with OM application where they could access more nutrients through reducing cluster-root formation in other places, indicating a high plasticity of root morphology to stimulation of OM application.

The inducible effect on cluster-root proliferation by OM could involve several processes related to nutrient release during breakdown of OM by micro-organisms. In *Hakea*, the high nitrogen content of OM increased non-cluster-root growth, but not that of cluster roots (Lamont, 1973). This suggests that nitrogen is not a major factor involved in cluster-root formation. However, NH$_4^+$-N stimulated cluster-root production in *L. albus* more than other nitrogen sources did (Sas *et al.*, 2002). NH$_4^+$-N in OM was about seven times higher than that in P-deficient soil (Table 1) which may partly explain the increased cluster-root formation in the OM layers. In contrast, P is a major factor for cluster-root formation (Dinkelaker *et al.*, 1995). The present study confirms that plants with a high shoot P concentration produced fewer cluster roots in any soil layers including the OM layer. Under P deficiency, localized application of OM induced cluster-root proliferation, and even phytate, as organic P, added to OM (T6) further increased formation of cluster roots, suggesting a positive effect of P released from organic P as well as from OM on cluster-root formation, which was associated with microbial activity. In accordance with this, the stimulating effect of OM on cluster-root formation disappeared when OM was sterilized, leading to cessation of OM breakdown by micro-organisms. In the present study, P released from OM or phytate was very limited and did not cause an increased P uptake or biomass of lupin plants. However, against the background of P-deficient soil, a slight increase of P supply in the localized OM layer significantly enhanced cluster-root formation, in agreement with previous evidence that formation of cluster roots in severely P-impoverished soil is enhanced by a slight increase in P supply, but strongly suppressed by a high P supply (Reddell *et al.*, 1997; Shane *et al.*, 2004). This positive effect of OM on cluster-root formation probably depends on the interaction of plant P status and localized supply of P in the soil substrate.

A significant difference in cluster-root distribution in the different layers was observed (Fig. 2). When OM was in the middle layer, the percentage of cluster roots in this layer in relation to total cluster roots of plants was up to 97%, except for the entirely sterilized treatment (T7). Organic matter in the top or middle layers increased the percentage of cluster roots compared with that in the corresponding layers of the homogeneous P-deficient treatment (T1). Under field conditions, cluster roots are also concentrated in the humus-rich surface soil horizons (Lamont, 2003). In the present study, the root distribution of *L. albus* in the OM layer was specific to cluster roots. There was no difference in proportion of cluster roots to total roots in dry biomass among no-P treatments (Table 4), independent of OM application, indicating that stimulation of OM of cluster-root formation was a local response. The results suggest that cluster roots tend to form in the zones where nutrients are likely to become available and can be accessed.

The relationship between formation and distribution of cluster roots, shoot P status and soil patches (OM and P) can be summarized as follows. Shoot P concentration regulates cluster-root formation of whole plants (Shane *et al.*, 2003; Shen *et al.*, 2005). P-deficient *L. albus* plants produce more cluster roots than P-sufficient plants (Neumann *et al.*, 1999; Shen *et al.*, 2005), with a good relationship between the number of cluster roots and shoot P concentration (Shane *et al.*, 2003; Shen *et al.*, 2003; Li *et al.*, 2008). Localized high P supplies can also suppress cluster-root formation through improving shoot P status (Shane *et al.*, 2003). Besides systemic signals, formation of cluster roots can also respond to the localized application of OM, localized P supply (Shane *et al.*, 2003; Shen *et al.*, 2005; Shu *et al.*, 2007) and even growth medium pH (Wang *et al.*, 2006) or physical properties (Shu *et al.*, 2005). When patches exist in the soil profile, particularly P-rich soil patches, cluster roots concentrate in these patches to facilitate P mobilization and uptake without changing the total proportion of cluster roots to total root biomass, indicating acclimation of cluster-root growth to environmental factors.

**Carboxylate exudation**

Cluster roots of *L. albus* exude large amounts of carboxylates into the rhizosphere, and citrate comprises a major...
fraction of all carboxylates (Gardner et al., 1983; Dinkelaker et al., 1989). Gerke et al. (1994) reported citrate concentrations in the cluster rhizosphere up to 55 mol g\(^{-1}\) soil. In the present study, the citrate concentration varied from 1-4 to 114 mol g\(^{-1}\) soil. The lowest concentration in the present study was observed in the treatments with P-sufficient soil; higher P availability decreased rhizosphere citrate concentrations. Localized application of OM did not change citrate concentrations. The result indicates that citrate concentrations in the rhizosphere are affected mainly by plant P status because P supply from OM was low in the present study.

Concluding remarks

Formation of cluster root of \textit{L. albus} was strongly stimulated by localized application of OM. Localized application of phytate plus OM as well as non-sterile OM significantly enhanced proliferation of cluster roots. The results show that OM shifted the distribution of cluster roots of \textit{L. albus} in the soil profile without changing the total amount of cluster roots. The positive effect of OM on cluster-root proliferation in the stratified system is most probably associated with P released from breakdown of OM or phytate, involving microorganisms. It is not related to a lower soil density and we have no evidence for microbial effects, other than those involved in releasing P.

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LITERATURE CITED


