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RESEARCH ARTICLE

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Sandy silt loam soil may hamper the inoculation effect on lucerne (*Medicago sativa* L.) growth

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ABSTRACT

Despite newly approved lucerne cultivars, this has not led to increased use of this legume in high-latitude agriculture. Challenges with inoculation by *Rhizobium meliloti* have been identified as a bottleneck to adaptation. Here we tested inoculation sources (ISs) with soil types and cultivars in pot and field experiments. During a one-year outdoor pot experiment, we tested the impact of IS (wet peat slurry and Nitragin Gold dry inoculation) and three soil types (sand, sandy silt and /peat soil) on nodule development, shoot and root growth and winter survival of one hybrid lucerne cultivar ('Ludvig'). The pot experiment revealed that dry inoculation led to significant better plant growth, flower and nodule development as well as plant regrowth after winter survival. Peat soil appeared as the best growth medium and silty soil limited inoculation efficiencies. In field trials at two locations differing in soil characteristics using similar ISs, and three hybrid lucerne cultivars ('Lavo', 'Live' and 'Lotte') biomass yield during two ley years showed site as well as cultivar differences. Such environmental interactions in the field trials justify the use of adapted cultivars, and dry inoculation should be recommended for practical use replacing peat slurry inoculation.

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Dry matter yield; nitragin gold; peat slurry inoculation; root nodules; rhizobia; shoot: root ratio

Introduction

Lucerne (blue lucerne, alfalfa, *Medicago sativa* L.) is the most widely grown forage legume in the temperate world (Sprent et al. 2017). It is a perennial autotetraploid ($2n = 4x = 32$) and is cross-pollinated by insects, predominantly bees (McCoy et al. 1988). Lucerne was introduced with good results in the Nordic countries many decades ago, especially under dry conditions (Wexelsen 1948; Lagerquist 1959); however, it has never reached the same level of use as red clover (*Trifolium pratense* L.) or white clover (*Trifolium repens* L.).

Forage legumes are important components in species mixtures because of their potential to increase productivity, forage nutritional value and resource efficiency (Peyraud et al. 2009; Suter et al. 2015; Ćupina et al. 2017). Lucerne, like other legumes, is especially important in organic farming and increasing the forage protein content. Although white and red clovers are preferred in legume-grass mixtures, red clover is generally perceived as having a low persistence in grassland leys. Therefore, an increasing interest in the inclusion of lucerne in species-rich leys has been recognised in recent years. Lucerne provides a valuable high-protein source but with moderate energy values due to low stem digestibility (Veronesi et al. 2010) and under favourable growing conditions exceeds red clover in yield and protein content (Sousa et al. 2020). Therefore, the inclusion of both clovers and lucerne in grass-legume mixtures can be complementary and may lead to a more stable proportion of legumes in leys over time.

Legumes contribute positively to ecosystem services. Increased storage of carbon (C) in grassland compared with annual crops has been demonstrated due to considerably higher root biomass in perennial species (Kätterer and Andrén 1999; Rasse et al. 2005), and the deep root system in lucerne can contribute positively to this effect. Climate change has been assessed as the major challenge for grassland-based farming in Europe. The increased variability in precipitation and temperature between years suggests that increased diversity of grassland mixtures is necessary to obtain resilient production systems (Huyghe 2020). More Northern

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regions of Europe are seeing higher temperatures, mainly during the winter period, resulting in a prolonged growing season. Resilience against climate change may be the most important factor, and here the introduction of new species of perennial legumes, such as lucerne and birdsfoot trefoil (*Lotus corniculatus* L.), has been recommended (Urruty et al. 2016).

Typically, lucerne is sensitive to harsh winter climates, which may restrict the use of lucerne in agriculture in marginal areas. The development of hybrid lucerne (*M. sativa* L./*M.*×*varia* Martyn) from crosses between blue lucerne and yellow lucerne (*M. sativa* ssp. *falcata*), in which yellow lucerne is the winter hardy parent (Mela et al. 1996), has improved its freezing tolerance considerably through breeding (Lunnan and Sturite 2015). Such new cultivars have certainly widened the potential growing area towards northern latitudes and higher altitudes in Europe. Seppänen et al. (2018) tested two hybrids and four blue lucerne varieties in Finland and found a hybrid cultivar as the most freezing-tolerant cultivar among the tested varieties. Varieties of hybrid lucerne have successfully been grown as far north as 65°N (Lunnan and Sturite 2015). Blue lucerne is drought-resistant, high yielding and more persistent than red clover; however, this depends on adequate levels of soil moisture, pH, nutritional supply and specific *Rhizobium* in place (Öhlund 2020).

Soils in high-latitude agriculture normally have no previous lucerne history. Therefore, the successful establishment of lucerne plants relies on commercially available seed inoculants containing symbiotic rhizobia. There is evidence that the best growth and nitrogen fixation of lucerne occur if the root nodulation is caused by the specific strain *Sinorhizobium meliloti* (Evans et al. 2005), which is widespread and common in soils on all continents (Sprent et al. 2017). The micro-organisms have, however, to be introduced into the soil in sufficient numbers to outcompete natural populations of ineffective rhizobia and to secure successful infection of legume roots (Bromfield et al. 1986). A long history of red and white clover cultivation has ensured that the necessary strains of *Rhizobium* for symbiotic N-fixation are present in Nordic pastoral soils. Thus, inoculation of clovers is necessary only when they are introduced in areas where they have previously not been present, e.g. in land reclamation. In contrast, there are no indigenous soil populations of *S. meliloti* in the soil necessary for successful lucerne growth, and populations of effective rhizobia, therefore, need to be built up through the introduction of commercial inoculation of the seed.

Peat slurry inoculation has been one of several options for establishing lucerne. The peat slurry inoculant is mixed with water and the seed is coated in the slurry mix and dried immediately before sowing. This method is widely used and favours a high nodulation rate and results in great herbage yield (Wigley et al. 2015; Jáuregui et al. 2019). Another option is the commercial practice of processing legume seed (lime-pelleting, Nitragin Gold where glue is used to stick inoculum to the seed), a method which has produced more reliable results and is superior compared to peat slurry inoculation in soils where the pH is below 5.5 (Stout et al. 1997; Deaker et al. 2004).

An easy and successful method of establishing lucerne is an important constraint in increasing the use of lucerne in high-latitude agriculture. In Norwegian farming, the peat slurry culture has, for some decades, been the main inoculant and marketed along with lucerne seed. However, on-farm experience over the last few decades, this inoculant has had reduced efficiency.

A range of soil chemical and physical factors influence the plant-soil-microbe environment. One of the key factors is soil pH (Chapparro et al. 2012) where lucerne has a critical soil pH of 6.2 (Goulding 2016). Other critical factors that affect lucerne establishment are soil type, water logging, oxygen availability and air permeability in the root area, or low effectiveness of the inoculated strain. There is limited knowledge about inoculation practices or methodology among farmers. Lunnan and Sturite (2015) concluded that proper drainage, high soil pH and successful inoculation were decisive for the establishment and forage production of lucerne.

The aim of this study was to test two inoculation sources (ISs): peat slurry inoculation (wet) and Nitragin Gold inoculation (dry) in an outdoor pot experiment with one lucerne cultivar and three growth media, and in field trials at two climatically differing locations testing three commercial cultivars.

The hypothesis of this study was that *effective inoculant and inoculation methods will increase nodulation and plant growth and will secure better winter survival and yield potential of lucerne in high-latitude agriculture*.

Materials and methods

ISs and methods

The peat slurry culture (*Rhizobium meliloti*, Prod. No. 012) is produced by Inocula Scandinavia (Hovmantorp, Sweden) and each bag contains approx. 1 billion cells g^{-1} which is sufficient for 10 kg seed. One portion was mixed with 500 ml water before being added to the seed 4–5 h before seeding, as recommended in the product declaration. For the field trial, the seed was inoculated at each experimental site before seeding.

The dry inoculation (Nitragin Gold®, Monsanto) contained 300 million viable cells of *S. meliloti* strain NRG-

185-1 g^{-1}. Inoculation with Nitragin Gold was performed at Fureneset for the pot experiment and the field trials and performed according to the recommendations for inoculation: mixing the seed directly with the dry powder from the carton at the rate of 189 g/22.7 kg of seed. The inoculation powder was supplied by DLF Trifolium, DK, and was stored under cool conditions with ideal storage conditions at 4–13°C before use.

Outdoor pot experiment

The pot experiment was performed at NIBIO Fureneset, Western Norway (61°18′N, 5°21′E, 10 m a.s.l.: coastal climate). Seeds of hybrid lucerne (cultivar 'Ludvig') were sown after being inoculated with (1) peat slurry (wet inoculation) and (2) dry powder of Nitragin Gold (dry inoculation). As a control non-treated seed was used. After inoculation, the seeds were sown in plastic trays with a similar amount of soil below and on top of the seeds to ensure no light exposition. Within the trays the soil was placed on top of paper towels to simplify necessary watering during the germination period. Thereafter the trays were maintained in darkness at a constant 20°C in a growth chamber until germination started.

Three growth mediums were compared: sand, sandy silt loam (silt) and peat. The sandy soil contained a mixture of medium (52%), fine (41%) and coarse sand (7%). The sandy silt loam soil (representative of coastal temperate and continental areas of Norway) was dominated by silt (55%), sand (34%) and clay (10%). The peat soil consisted of 100 vol.% Sphagnum, H2–H4 (von Post's scale; 1922). The content of the most important nutrients was analysed before the start of the experiment at Eurofins (Table 1). Based on these analyses and before starting the experiment, phosphorus (P) and potassium (K) were added to the sandy and silty soils to avoid deficiency compared to peat soil. Fertiliser was added 47 kg ha^{-1} of P and 177 kg ha^{-1} K (PK 5-17, ®Yara). Lime was added to the same two soil types using coarse dolomite (containing 11% Mg) to ensure the sufficient availability of magnesium (Mg). The neutralising value in the coarse dolomite was 38/50 wt.% of calcium oxide (CaO) after one and five years, respectively. To increase the pH to 0.2 pH-units in the sandy soil and 0.1 pH-unit in the silty soil, 1300 kg ha^{-1} of coarse dolomite was added to the sandy and 900 kg ha^{-1} to the silty soil. Nitrogen (N) (75 kg ha^{-1}) in the form of calcium nitrate (15.5% NH_4NO_3 (85% NO_3-N and 15% NH_4-N)) was added to sandy and silty soils to compensate for the N-amount in the peat soil.

Ten days after inoculation and sowing, the seedlings were transferred to 10 L pots either with sand, silt or peat soil. In each pot 12 plants were planted and grown. In total, eight pots with three replicates per treatment were established of which six pots were used for destructive sampling during the growing season, plants were chosen randomly from those pots at each sampling date and two pots per treatment were left unharvested for the assessment of winter survival. The plants were fertilised regularly during the growing season with 5 g of PK and 2.5 g of calcium nitrate ($Ca(NO_3)_2$) per pot (0.05 m^2/pot) and watered when needed. Destructive plant sampling took place 46 (sd1,), 60 (sd2), 74 (sd3), 88 (sd4), 102 (sd5), 116 (sd6), 130 (sd7) and 144 days (sd8) after the plants were transferred to pots on 3 July 2017. On each of the eight sampling occasions, a replicate of three plants per IS and soil type were carefully dug up and investigated, in total 27 plants per sampling date. At each sampling, plant shoot height (cm) from base to the bud or flower end, number of buds/flowers and number and type of nodules were determined. Single nodules were counted; however, with time the nodules formed clusters of nodules (groups of nodules that cannot be teased apart). The clusters were counted and divided into small, medium or large clusters. The root fraction of the sampled plants was thereafter transferred to individual plastic bags and frozen until further analysis. Frozen roots were allowed to thaw at room temperature for 24 h and then washed and scanned with an interactive scanner-based image analysis programme WinRHIZO (Regent Instruments Inc. 2017) for total root length (cm) and root volume (cm^3) at all sampling dates except sd5 and sd7. After root scanning the root biomass was dried at 60°C for 48 h to estimate the dry matter biomass.

The pots with remaining plants and unharvested pots were hardened in natural conditions and kept out during winter. In spring 2018, the number of surviving plants was counted and by June 2018 all plants were cut at 3 cm height, and the green biomass was dried at 60°C for 48 h for the estimation of dry matter biomass per pot. Dry matter per surviving plant was estimated as the ratio between dry matter and the number of surviving plants per pot.

Field trials

Two field trials were established in 2017, one at NIBIO Løken (61°04′N, 09°04′E inland, 520 m a.s.l.) established on 15 June 2017 on silty medium sand and one at NIBIO Tjøtta, Northern Norway (65°49′N, 12°25′E coastal, 11 m a.s.l.) on 26 May 2017 on sandy soil formed since the last period of glaciation until about 10,000 years ago and classified as medium sand. Soil

Table 1. Description of the soil characteristics for the pot experiment and the soil at Tjøtta and Løken.[a]

	Pot exp., Fureneset			Field trial		
Site/soil type	Sandy soil	Silty soil	Peat soil	Tjøtta	Løken	Method
Parameters and units						
Volume weight (kg L^{-1})	1.7	2.0	<0.1	1.3	1.1	
pH	6.1	6.2	5.8	8.1	5.5_a	ISO 10390
Phosphorus (P-AL) (mg/100 g)	<2.0	3.2	67	17	12	AL[b]
Potassium (K-AL) (mg/100 g)	<2.0	7.3	190	3.7	4.1	AL
Magnesium (Mg-AL) (mg/100 g)	<1.0	12	110	110	9.1	AL
Calcium (Ca-AL) (mg/100 g)	<10	58	1200	>2000	100	AL
Sodium (Na-AL) (mg/100 g)	<5.0	<5.0	24	31	<5.0	AL
K-HNO_3 (mg/100 g)	68	170		37	52	
Loss of ignition (% dry matter)	0.50	1.3	89.4	4.5	6.2	

[a]Soil pH was measured at Løken in 2016, 6 t ha^{-1} of course dolomite was applied in spring 2017; [b]AL -ammonium lactate.

parameters for both sites are shown in Table 1. At Tjøtta a supplementary trial was established for destructive plant sampling in the first ley year. In the second ley year, the supplementary trial was included in the assessment of plant survival and yield measurement. On both locations there had been lucerne cultivation previously, but not at the actual field trial sites.

Three cultivars of lucerne (cvs. Live, Lotte and Lavo, Graminor Ltd. (2002), Hamar, Norway) were inoculated as described previously for the pot experiment that included wet and dry inoculation, and uninoculated seeds used as control. At Løken, 6 t ha^{-1} of course limestone was applied in spring 2017, and annual fertilisation was 30 kg P ha^{-1} and 120 kg K ha^{-1}. The field trials at Tjøtta received annually 40 t ha^{-1} cattle slurry in spring (80 kg N ha^{-1}, 36 kg P ha^{-1} and 88 kg K ha^{-1}) and an additional 5 kg P ha^{-1} and 20 kg K ha^{-1} after the first cut. Plots, 1.5 × 7 m, were established in a randomised block design with three replicates of each treatment. Winter survival and seasonal biomass production were assessed. The herbage from each plot was harvested and weighed using a Haldrup plot harvester (Haldrup, Løgstør, DK). At both sites, two cuts were taken during the growing season and subsamples of the plot herbage were dried at 60°C for 48 h to estimate dry matter yield (DMY). To assess nodule activity at the Løken site, the greenness of the plant stand at each plot at first cut was visually scored using a scale from 1 to 9 (9 is dark green).

In 2018 from the field trial for destructive plant sampling at Tjøtta, three plants per plot were carefully excavated twice a month. In total, six field sampling dates (fsds) were performed on 14 June (fsd1), 25 June (fsd2), 11 July (fsd3), 26 July (fsd4), 7 August (fsd5) and 30 August (fsd6). These three plants were pooled together and made one of three replicates per IS and cultivar, in total at least 90 plants per sampling date were excavated. Plant height and the number of buds and flowers were measured similarly as in the pot experiment immediately after sampling. The roots from each plant were kept frozen as in the pot experiment and later scanned with WinRhizo. The nodule counting function at WinRhizo was used to determine the number of single nodules and the clusters of nodules in the scanned image. Both above and below plant fractions were dried at 60°C for 48 h for the estimation of dry matter biomass and shoot:root ratio. The trial for destructive sampling was cut once on 27 June during the growing season.

Weather data

Temperature (°C), precipitation (mm) and the destructive sampling period for the experimental period May 2017–August 2019 are shown in Figure 1 for the three sites (data are taken from on-site weather stations). The long-term mean annual precipitation for the standard period 1991–2020 is 2301 mm at the coastal south site Fureneset, 1321 mm at the northern coastal site Tjøtta and 630 mm at the high-altitude inland site Løken. At Fureneset and Tjøtta the precipitation is predominantly rain, whereas at Løken the period of snow cover is about five months. Similarly, the mean annual temperature is 7.7°C at Fureneset, 6.3°C at Tjøtta and 2.9°C at Løken.

The mean temperature at Fureneset for June–October 2017 was 12.5°C, and the total precipitation was 1047 mm, and similarly, for November 2017–March 2018 was 1.8°C and the total precipitation was 1134 mm. The temperature for the growing period May–August 2018 was 14.4°C and 12.4°C for Løken and Tjøtta, respectively, and similarly 11.9°C and 12.2°C for 2019 for Løken and Tjøtta, respectively.

Statistical analyses

A fully randomised block design was used for the pot experiment. The effects of sampling date, soil type and IS on the plant parameters were calculated and their statistical significance was tested using the general

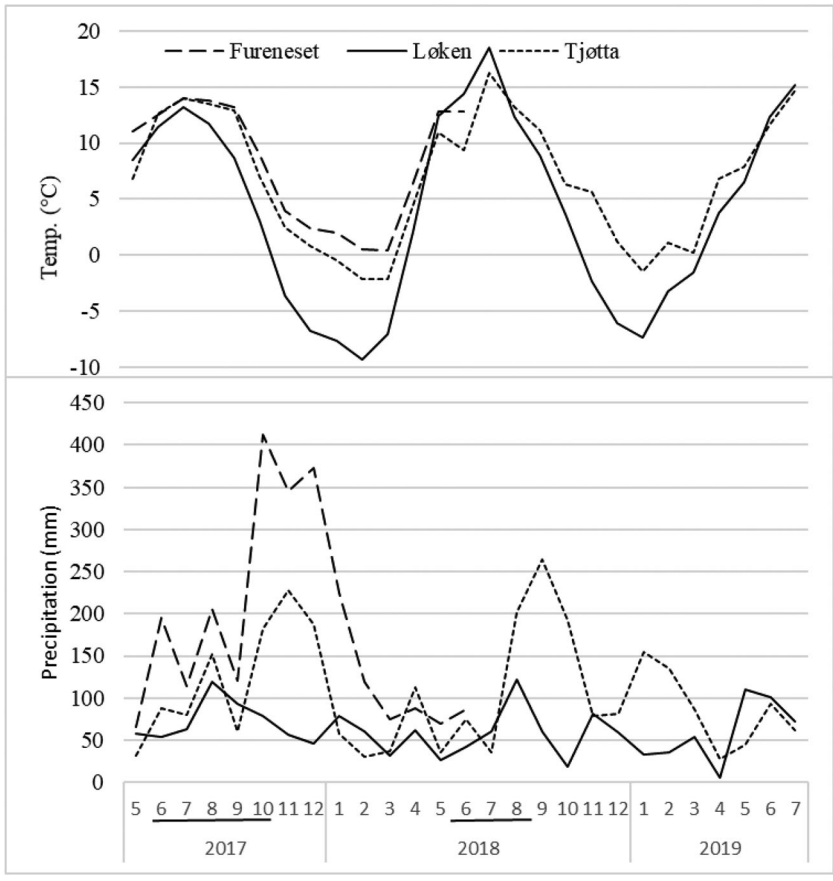

Figure 1. Mean monthly air temperature (°C) and precipitation (mm) at the coastal experimental site (Fureneset, pot experiment) during late May 2017–June 2018 and for the two field trial sites (Løken and Tjøtta) for the three-year study period. Destructive sampling dates at Fureneset in 2017 were from June to October (months 6–10) and at Tjøtta in 2018 from June to August (months 6–8).

linear model (GLM) (SAS 9.4; SAS Institute Inc., Cary, NC, USA) where sampling date, soil type and ISs were considered as fixed factors. For the field experiments at Løken and Tjøtta, the effects of location, ley year, IS and cultivars were tested using the GLM where location, ley year, IS and cultivars were considered as fixed factors. During statistical analyses, either cultivars or ISs were tested at one time. For the plant fractions at Tjøtta, the effects of IS and cultivars were tested using the GLM where IS and cultivars were considered as fixed factors. Differences between parameters were tested by Tukey pairwise comparisons.

Results

Outdoor pot experiment

Plant growth characteristics

Plant shoot height increased rapidly from the first sampling date (sd1, 27.2 cm) until sd4 (49.1 cm) and then evened out over the last four sampling dates to 56.0 cm on average across all treatments. Sampling time, IS and soil type all affected plant shoot height ($P < .001$; Table 2). Dry inoculation of the seeds accelerated plant growth, and on average for all sampling dates plant height in dry inoculated treatments was significantly greater (50.5 cm) than for uninoculated (44.4 cm) and wet inoculated seeds (43.3 cm; Figure 2). The plants grown in peat were significantly taller (73.4 cm; $P < .001$) than the plants grown in sandy soil (35.2 cm) or silty soil (29.8 cm; Figure 3).

Flower development during eight sampling dates was also affected by sampling time, IS and soil type (Table 2). Plants produced significantly ($P < .001$) more flowers with dry inoculated seeds than with wet inoculated and uninoculated seeds (Figure 2). Flower development started earlier when lucerne was grown in peat soil (Figure 3). This resulted in significantly ($P < .001$; Figure 3) more flowers per plant on average across all sampling dates in peat soil (1.4 flowers) than in sandy (0.4 flowers) or silty soil (0.2 flowers). A sampling date and soil type interaction were due to small differences in the ranking of sampling dates when testing each soil type separately.

Table 2. Probability levels of analysis of variance (ANOVA) models testing the effects of shoot height (cm), numbers of flowers, single nodules, clusters of small, medium and large nodules, root length (cm), root volume (cm^2) and root biomass (g dw) for sampling date, inoculations source, soil type and factor interactions and mean estimates of the parameters. The numbers next to the parameters show the number of replicates.

Parameters	Sample date (SD)	IS	Soil type (soil)	SD * soil	SD * Is	Soil * IS	SD * soil * IS	Mean
Shoot height (cm) 215	<.001	<.001	<.001	.009	ns	ns	ns	46.01
Flower number 215	<.001	<.001	<.001	<.001	ns	.048	ns	0.67
Nodules (single) 215	<.001	<.001	<.001	ns	ns	.003	.008	6.21
Small nodule cluster 215	<.001	<.001	<.001	.014	.005	<.001	.028	6.65
Medium nodule cluster 215	<.001	<.001	<.001	.004	ns	<0.001	ns	1.84
Large nodule cluster 215	.002	ns	.042	.044	ns	ns	ns	0.61
Root length 128	<.001	ns	<.001	<.001	ns	ns	ns	2085
Root volume 128	<.001	.054	<.001	.0005	ns	ns	ns	2.31
Root biomass (g dw) 116	<.001	ns	<.001	<.001	ns	ns	ns	1.29

Nodulation with single nodules was affected by sampling date, IS and soil type (Table 2). The number of nodules increased from the first sampling date throughout the growing season (Figure 4). On average across all sampling dates significantly ($P < .001$; Figure 4) more nodules were counted on roots inoculated with dry inoculate (11.2 nodules) compared with wet (4.2 nodules) and uninoculated seeds (3.3 nodules). Similarly, more nodules were found in the peat soil than in the sand and silt soils ($P < .001$; Figure 4). The soil and IS interaction was due to different ranking of nodule development in soil types when studying the ISs separately. Both the dry and wet inoculation increased the number of nodules in the following order peat > sand > silt, whereas for uninoculated plants the ranking was sand > silt > peat.

Small, medium and large nodule clusters appeared at sd2, sd3 and sd4, respectively. The clusters in all sizes

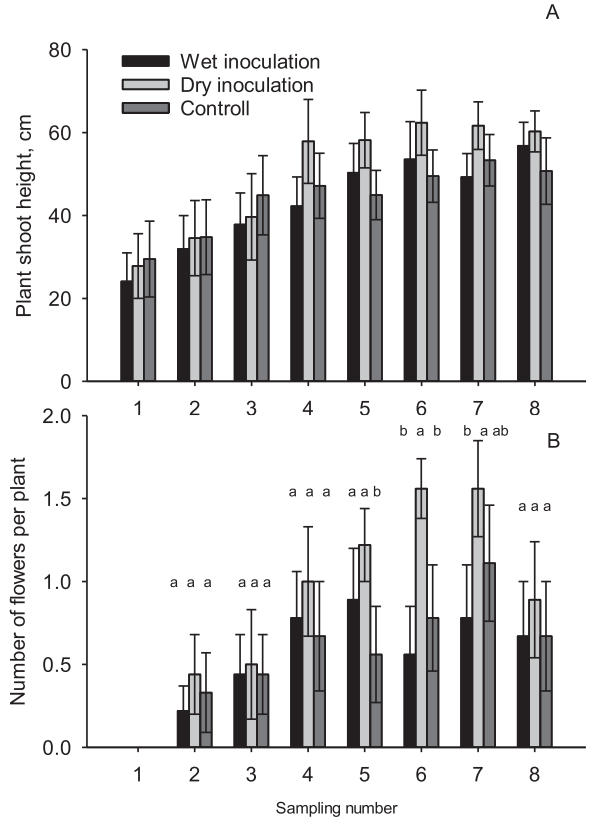

Figure 2. Plant shoot height (cm; A) and number of flowers per plant (B) on eight destructive sampling occasions in the pot experiment at Fureneset showing the effect of ISs (wet, dry inoculation and control). Vertical bars represent the standard error of the means. Different letters indicate the significances between wet and dry inoculation and the control.

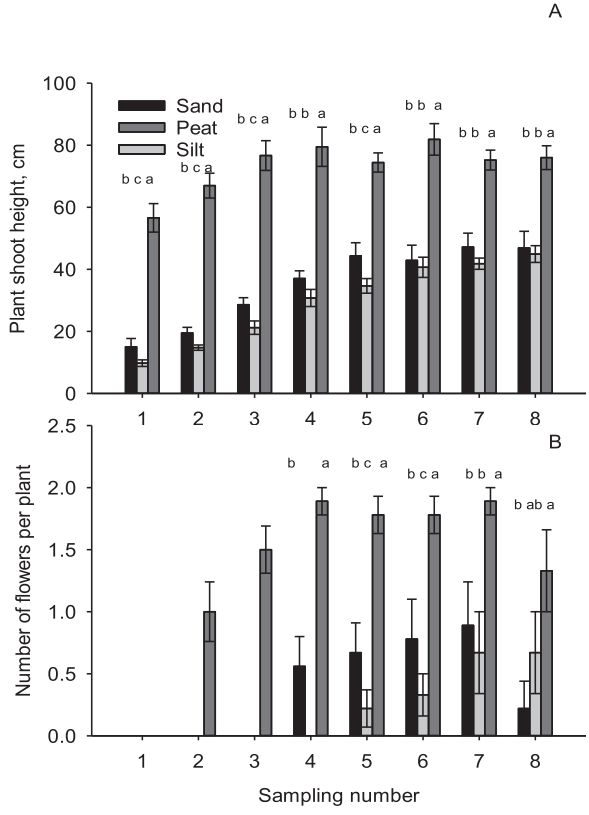

Figure 3. Plant shoot height (cm; A) and number of flowers per plant (B) on eight destructive sampling occasions in the pot experiment at Fureneset showing the effect of soil type (sand, silt and peat). Vertical bars represent the standard error of the means. Different letters indicate the significances between sandy, silty loam and peat soils.

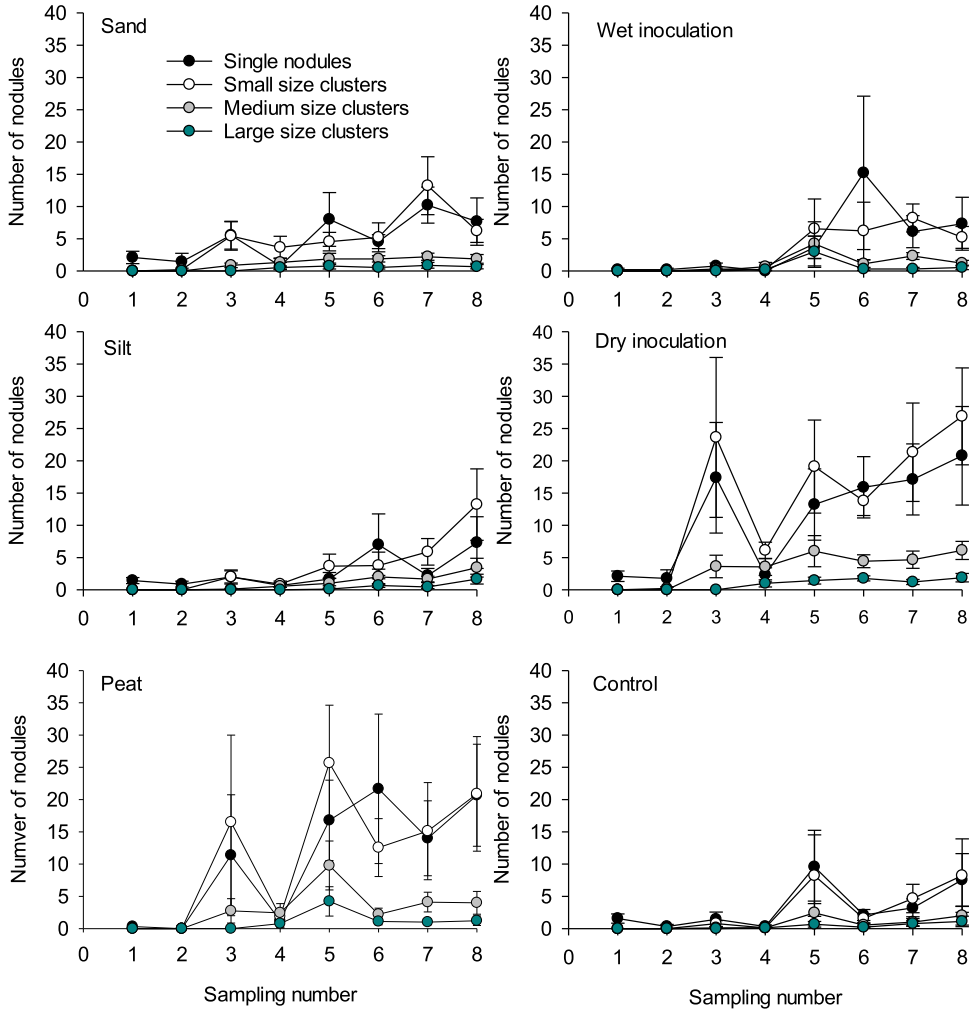

Figure 4. The number of single nodules and nodule clusters (small, medium and large) as the effect of soil type (sand, silt and peat) and IS (wet and dry inoculation and control) during eight sampling dates (1–8) from early June to early October 2018 (pot experiment, Fureneset). Vertical bars represent the standard error of the means.

increased in number until the last sampling date, but in several cases, the maximum number of clusters was reached on the fifth or sixth sampling date (Figure 4). All size groups were influenced by sampling date and soil type and also IS, except for large clusters (Table 2). For all three groups, significantly ($P < .001$) more clusters were found on lucerne roots treated with dry inoculate than wet and untreated seeds. However, for large size clusters, there were no differences between treatments. Peat soil accelerated cluster development significantly ($P < .05$; Figure 4) more than sandy and silty soil.

Lucerne root length and root volume were both affected by sampling date and soil type (Table S1). On average for all sampling dates, the root length in peat was 7350 cm and it was significantly ($P < .001$) greater than the root lengths in sandy and silty soils, which were 2881 and 1068 cm, respectively. Similarly, root volume was significantly greater in peat than in sand and silt soil. The root biomass increased significantly ($P < .001$; Table 2) from sd1 (0.1 g dw plant^{-1}) to sd8 (3.3 g dw plant^{-1}) and was influenced by sampling date and soil type. Root growth in peat soil was significantly greater than in the other two soil types ($P < .001$). The IS did not affect root biomass (Table 3).

Winter survival and spring growth

The survival rate of plants grown in peat was significantly higher than for plants grown in either sand or silt soil ($P < .001$). The number of plants that survived in peat soil was twice that of the other two soil types: 8 and 4 plants pot^{-1}. A similar pattern was recorded for spring regrowth. The lucerne grown in peat produced significantly greater plant biomass than plants grown in sand and silt soil ($P < .001$; Table 4). The dry inoculation contributed to significantly better plant regrowth in spring than plants treated with wet inoculation or uninoculated plants ($P < .001$; Table 4).

Table 3. Averaged across sampling dates root tissue biomass in pot experiment as affected by soil type and IS. The number of replicates is shown in brackets.

	Soil type			IS		
	Sand (54)	Silt (54)	Peat (53)	Peat slurry (54)	Dry (53)	Control (54)
Root tissue, g dw plant^{-1}	$0.75 \pm 0.1_{b}$	$0.51 \pm 0.1_{b}$	$2.64 \pm 0.3_{a}$	$1.19 \pm 0.2_{a}$	$1.45 \pm 0.3_{a}$	$1.24 \pm 0.3_{a}$

Note: Different letters in rows (within soil type and IS) indicate significant differences between soil types or between ISs.

Field trials

Effects on DMY and cultivars

On average over two ley years the DMY was significantly ($P < .001$) greater at Løken than at Tjøtta, 7.06 and 4.84 t ha^{-1}, respectively (Table S2). The DMY increased significantly from the first to the second ley year ($P < .001$; Table S2) and this increase between ley years was seen at both sites, however, with a higher increase at Løken.

Overall, cv. 'Lavo' yielded significantly more than the cvs. 'Lotte' and 'Live' ($P < .001$; Table S3). The cv. 'Lavo' yielded significantly more than the two other cultivars at the northernmost site, Tjøtta ($P < .04$), whereas at Løken cvs. 'Lotte' and 'Lavo' yielded significantly more than cv. 'Live' (Tables 5 and S4).

In field trials, no effect of the IS on yield was recorded, except for cv 'Lavo' at Løken (Table 5). However, in the plots where seeds were treated with dry inoculate DMY tended to be the greatest (Table S3). A visual evaluation of plant greenness at the first cut at Løken showed differences between ley years. The plant greenness intensified from the first ley year to the second year of harvest significantly ($P < .001$). The lucerne cv. 'Lotte' treated with dry inoculate scored the highest value (6.8) and it was significantly higher than for cv. 'Live' in wet treatment (4.8; $P < .001$).

Plant fractions and root development

Overall treatments at Tjøtta, the plant shoot height increased from 30.2 cm to 37.7 cm until the cut in late June. Two weeks after the cut the plant shoot height was on average 14.8 cm and in late August at fsd6 the plant shoot height attained 46.6 cm (Figure 5(B)). Here, wet inoculated seeds accelerated significantly better plant growth than uninoculated plant seeds ($P < .05$). Buds and flowers were recorded from the beginning of August (fsd5; Figure 5(B)).

Plant shoots, dry biomass increased following the June cut after fsd2 until it reached the peak at fsd5 of 52.94 g plant^{-1}. At the last sampling date (fsd6) some reduction in the plant shoot biomass was recorded. On average for all cultivars and ISs, root dry biomass showed a rather even root development from fsd1 to fsd4. Considerably, root biomass increase was measured in fsd5 and fsd6, 0.89 and 1.37 g plant^{-1}, respectively.

The shoot:root ratio reflected the cut effect in June and showed a considerable increase from fsd3 to fsd5 (Figure 5(A)). A reduction in the shoot:root ratio in fsd6 was due to the high root growth in late August (Figure 5(A)).

On average for all treatments, the numbers of single nodules increased until the fsd4 in late July before decreasing (Figure 5(C)). The number of single nodules in fsd4 was 21.0 plant^{-1} and this was significantly more than in all other sampling dates. Sampling time, IS and cultivar had no effect on cluster size nodules developed (Figure 5(C)).

Discussion

Increasing the growth area of lucerne in high-latitude agriculture depends on successful inoculation of the adapted strain of *S. meliloti* to secure good establishment and growth. In this study, the response of dry inoculation was significant only in the pot experiment, and the results from the pot experiment confirmed our hypothesis for increased DMY and winter survival together with good nodulation. In the field experiments, the dry inoculated seeds tended to produce greater DMY than other treatments; however, this effect was without significance. At the same time, we recognised

Table 4. Mean spring growth of surviving plants in pot experiment as affected by soil type and IS. The number of replicates is shown in brackets.

	Soil type			IS		
	Sand (23)	Silt (22)	Peat (24)	Peat slurry (22)	Dry (23)	Control (24)
Spring growth, g dw plant^{-1}	$1.76 \pm 0.21_{b}$	$1.62 \pm 0.26_{b}$	$9.24 \pm 0.42_{a}$	$3.67 \pm 0.73_{b}$	$5.28 \pm 0.82_{a}$	$3.99 \pm 0.87_{b}$

Note: See the table 3.
Different letters in rows (within soil type and IS) indicate significant differences between soil types or between ISs.

Table 5. Mean annual DMY (t ha^{-1}) for two ley years of three cultivars (Lavo, Lotte and Live) and two ISs (dry and wet) and non-inoculated (control) across both locations at Løken and Tjøtta separately. The number of replicates (N) and coefficient of determination (R^2) are added.

Cultivars	Lavo[A]			Lotte[B]			Live[B]		
Inoculation	Dry	Wet	Control	Dry	Wet	Control	Dry	Wet	Control
All trials: $N = 15$ $R^2 = 0.87$	6.39 ±0.48 a	6.01 ±0.47 a	5.98 ±0.43 a	5.69 ±0.57 a	5.64 ±0.51 a	5.50 ±0.51 a	5.38 ±0.47 a	5.34 ±0.51 a	5.61 ±0.53 a
Løken: $N = 6$ $R^2 = 0.95$	7.74 ±0.47 a	7.18 ±0.75 ab	6.73 ±0.81 c	7.53 ±0.80 a	7.00 ±0.75 a	7.18 ±0.84 a	6.37 ±0.90 b	6.27 ±0.90 b	7.52 ±0.68 a
Tjøtta: $N = 9$ $R^2 = 0.44$	5.49 ±0.57 a	5.23 ±0.46 a	5.49 ±0.44 a	4.47 ±0.44 a	4.74 ±0.53 a	4.39 ±0.26 a	4.71 ±0.43 a	4.73 ±0.55 a	4.34 ±0.35 a

Notes: Means in rows separately for each cultivar followed by different letters are significantly different at the 5% level. Small letters denote differences between inoculation treatments within each cultivar and capital letters denote differences between cultivars.

response differences between the tested cultivars (Tables 5 and S3). There was, however, a sizeable interaction to soil types and characteristics as well as cultivars.

Soil type and soil properties

In the pot experiment, the peat soil returned the greatest growth in the above- and below-ground plant fractions as well as nodulation rate and size (Table 2). Sphagnum peat (H2–H4) has a good structure facilitating a large pore volume with a high proportion of large pores. Such soil properties with high air volume and permeability in the upper soil horizons promote root and nodule development. This is in contrast with the small pores dominating the clay-holding sandy silt loam, where the air volume and permeability are low due to high water capillary capacity (Lipiec et al. 2006). Differences in soil characteristics were especially pronounced after the winter period in spring where the pots containing sandy silt loam soil stayed waterlogged, while other soil types drained well. The high bulk density measured in silty sand also indicated limited availability to oxygen and consequent low nodulation rate and plant growth in sandy silt loam soil (Figure 4). Thus, soil properties such as bulk density, air permeability and water availability seem to be important in successful lucerne establishment.

Soil pH level

A high soil pH is a prerequisite for successful lucerne establishment and growth. In our pot study and the field trial at Løken, the soil pH was around 6 which for forage production is optimal. The pH 8 found in the field at Tjøtta is due to the subsoil originating from marine shell sand in this region. The obtained pH levels are generally higher than the pH level found in soils in high-latitude agriculture. The wet peat slurry inoculant used in our studies requires an optimal soil pH close to 7 (Bo Falk, Inocula Scandinavia, manufacturer of the peat slurry inoculant). The efficiencies of the bacterial strain may be reduced when the soil pH is lower than the optimal pH level since the bacteria may use energy or reserves to maintain activity. Decreasing pH below 6 in Canadian soils caused decreased numbers of *R. meliloti* bacteria and nodulation rate (Rice et al. 1977). The use of lime-coated pre-inoculated seeds has extended the possibilities to grow lucerne in soils with lower pH, and good root nodulation of lucerne in soils with pH 5.1–5.9 has been reported (Rice and Olsen 1983; Stout et al. 1997). These studies partly explain why dry inoculation resulted in higher nodulation rates in the pot experiment and greater DMY than wet inoculation for particular cultivars in a field trial at Løken (Figure 2). A minor suboptimal soil pH at around 6 in the pot experiment may have revealed the potential differences caused by ISs. The smaller differences in response to IS in the field trial at Tjøtta are possibly the result of the high soil pH and very good conditions for establishment.

At Løken, differences in the greenness of the plants were visible in the first cut the year after seeding. In the regrowth period, most of the plants became deep green (data not shown), indicating that over a year was needed to achieve high nodule activity. A similar delay in nodule activity was not observed at Tjøtta.

Increased precipitation with more intense periodic events during the last decades has caused soil acidification, which is not favourable for a drought-tolerant bacteria strain with its sensitivity to precipitation. Generally, liming of agricultural land has been reduced and does not compensate for the use of ammonium(NH_4-)-based fertilisers, which acidify soils through the nitrification process (Aasen 1977; Riley 2007; Goulding 2016). For much of the grassland area in these regions, the soil pH may be closer to 5 than 6, which suggests that liming on a grand scale must be carried out. Stout et al. (1997) found that liming in combination with inoculation increased lucerne DMY by 130%. It should, however, be considered that the soil pH requirement increases with the increased content of silt and clay

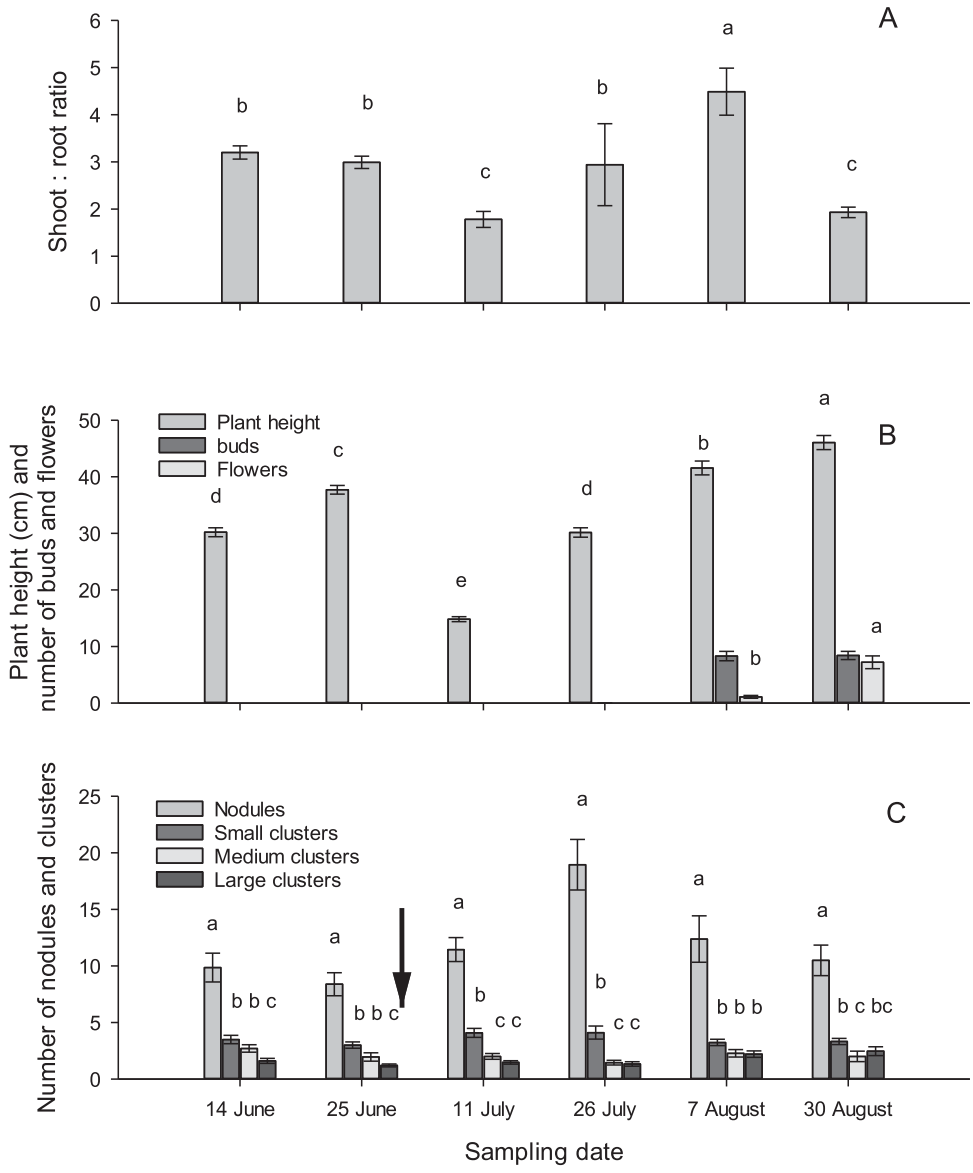

Figure 5. Plant shoot:root ratio (A), plant height (cm), number of buds and flowers (B) and number of single nodules and small, medium and large nodule clusters (C) during six destructive sampling times in the field experiment at Tjøtta. The date of cut was 27 June 2018 (shown by arrow). Vertical bars represent standard errors of the means. Different letters indicate significances in the shoot:root ratio between sampling times (A), plant height and the number of flowers between sampling times (B) and the number of nodules and clusters within sampling time (C).

(Fleming and Foy 1968; Semb 1977). Based on our pot experiment, soil with high content of organic matter can provide acceptable growth of lucerne at lower soil pH. Accelerated growth of lucerne in peat soils can also be potentially due to the low levels of aluminium.

Environmental impact on lucerne's growth

Our results indicated environmental interactions with cultivars. At the Løken experiment, cv. 'Lavo' proved most winter hardy and the most productive. At Tjøtta, cv. 'Lavo' showed considerably higher DMY than cvs. 'Lotte' and 'Live', however, with no statistically significant differences due to high variation in the field (Table 5). This confirms results from field experiments in Finland where cv 'Lavo' showed good winter hardiness and yield benefit (Seppänen et al. 2018).

Such pronounced environmental interactions indicate that there is a potential market for an increase in the number of cultivars adapted to different climatic and management regimes. Our results suggest that for optimal production in high-latitude agriculture, good winter hardiness combined with well-matched inoculants for specific cultivars should be further studied. However, the requirements relating to the amount and effectiveness of the marketed inoculants are according

to Deaker et al. (2004) depending on the regulatory bodies in different countries.

Infection of nodules was recorded in treatments with uninoculated seed both in pot and field experiments, while a small degree of contamination during the establishment of the field trials, e.g. via the seed drill or soil transport during rainfall could be responsible. However, there is evidence that lucerne roots can be colonialised by natural strains and species of *Rhizobia*. Wigley et al. (2015) found that plants from uninoculated seed were nodulated by the *Rhizobium* sp. genotype A and that the lucerne nodules treated with *S. meliloti* in the form of peat slurry contained approximately 30% *Rhizobium*. Another New Zealand study showed that nodules from bare plants contained 11 bacterial genotypes exceeding those in the inoculated plants (Jáuregui et al. 2019).

Conclusion

The establishment of lucerne as a key legume in high-latitude agriculture is an important goal; however, there are several constraints to successful establishment. These are achieving plant inoculation with suitable symbionts and establishing optimal soil conditions, especially soil pH. Although natural strains of *Rhizobia* may provide sufficient inoculation for the development of lucerne plants, there is little information on the populations and community structure of such strains in the boreal soils and this needs to be addressed. Therefore, to ensure consistent nodulation an external source of inoculation is required where lucerne has not been previously cultivated and even the field experiments showed an unsignificant inoculation effect. Our pot experiment has demonstrated that the best approach would be to use a dry inoculation with a source of an *S. meliloti* bacterial strain using pre-coated seed. This approach coupled with improving soil pH could be transformational for the practical farming of lucerne.

Notes on contributors

Ievina Sturite is a research scientist at NIBIO Division of Food Production and Society who has worked on many topics related to grasslands and nutrient turnover in the plant-soil system. She is currently involved in several projects related to carbon storage in agricultural soils.

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Disclosure statement

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