# Assisted Revegetation in a Subarctic Environment: Effects of Fertilization on the Performance of Three Indigenous Plant Species

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## **Abstract**

Assisted revegetation is particularly difficult in subarctic and arctic ecosystems where the impact of anthropogenic activities can be extensive and natural plant regeneration is slow. The construction of a military base in the 1950s at Kuujjuarapik–Whapmagoostui in northern Quebec destroyed most of the vegetation cover. Afterwards, other anthropogenic disturbances linked to the village expansion (housing, ATV traffic, pedestrian trampling) have slowed down the recovery process. To provide residents with low-cost but efficient assisted revegetation techniques, we evaluated the performance (seedling emergence, survival, and biomass production) of three indigenous plant species (Leymus mollis, Lathyrus japonicus, Trisetum spicatum) submitted to different levels of mineral and organic fertilizer additions in both a greenhouse experiment and a field plantation in the village. In the greenhouse experiment, moderate mineral fertilization had positive impacts on seedling emergence and both aboveground and belowground biomass of L. mollis. The magnitude of this impact on biomass was greater when mineral fertilization was combined with organic fertilization. The effects of mineral fertilization were negative on the other two species, especially at higher fertilization levels. However, after two growing seasons, a moderate level of mineral fertilizer in the field plantation had positive effects on the cover and aboveground biomass of all three species. Overall, organic fertilization from the substrate of a nearby marsh did not enhance plant performance in either experiment. Planting seeds of L. mollis or T. spicatum in combination with a moderate level of mineral fertilization at the time of planting provides a low-cost assisted revegetation treatment for subarctic villages.

DOI: 10.1657/1938-4246-41.4.434

## Introduction

In arctic and subarctic regions of North America, anthropogenic pressure from the modern settlement of communities, exploitation of mineral and hydrological resources, and the development of ecotourism ventures led to the progressive degradation of pristine environments during the 20th century. Because of its negative impacts on the fragile ecosystems of the North, some northern communities are considering ways to mitigate this rapid development. Natural revegetation following such disturbance can be very slow in this region (Harper and Kershaw, 1996; Forbes et al., 2001) and restoration efforts have been minimal due to the lack of proper restoration guidelines. According to Forbes and McKendrick (2002), ecological restoration is still in its early stages in much of the subarctic and arctic zone due to a limited understanding of fundamental ecosystem processes, although some progress has been made recently. Experiments are then essential in order to determine what measures need to be taken to restore native species in such harsh disturbed environments.

Natural revegetation at sites disturbed by anthropogenic activities in subarctic ecosystems is subject to a harsh climate that reduces primary productivity. Sub-optimal temperatures and the short duration of the growing season slow down the pedogenic processes associated with soil development in arctic ecosystems (Kershaw, 1983; Harper and Kershaw, 1997), resulting in soil with low nutrient availability (Van Cleve and Viereck, 1981). Moreover, nutrients stocked in plant biomass or in the superficial layers of the soil are often exported from the ecosystem during or shortly after the disturbance. As a consequence, low nutrient availability negatively affects the rate of biomass accumulation. In this context, fertilizer addition during restoration can overcome the negative impacts of low nutrient availability. While the addition of mineral fertilizer only increases nutrient availability, the addition of organic fertilizer can also increase ion-exchange capacity (reducing nutrient leaching) and water retention capacity (Johnson, 1987; Elmarsdottir et al., 2003). Regeneration of indigenous plant cover can also be inhibited by factors common to all regions, including the absence of viable seeds (Ebersole, 1989; Forbes and Jefferies, 1999; Prach et al., 2001) or of mycorrhizal inoculum (Greipsson and El-Mayas, 1999; Blanke et al., 2007), herbivory (Arnalds, 1987; Bradshaw, 1987), substrate instability (Walker and Del Moral, 2003), compaction (Billings, 1987), desiccation (Arnalds, 1987; Cargill and Chapin, 1987; Chapin, 1993), and low nutrient availability (Arnalds, 1987; Bradshaw, 1987; Blanke et al., 2007).

The northern village of Whapmagoostui, in subarctic Québec is a typical example of a site that has undergone numerous anthropogenic disturbances in the last 50 years (Desormeaux, 2005). Originally an outpost for the fur trade in the earlier part of the 20th century, the vegetation prior to 1950 was almost continuous and covered 306 hectares. It was dominated mainly

by deciduous shrub, herb, lichen, and moss species. The construction of a military base in 1955 destroyed 56% of the vegetated area (ca. 172 hectares). Afterwards, vegetation recovery has been slowed down by different urbanization waves and increasing disturbance linked mainly to heavy trampling (pedestrian, all-terrain vehicles (ATVs) and other means of transportation). In 2001, vegetation cover was estimated to be 222 hectares (Desormeaux, 2005), although several residual vegetation islands in the village were seriously threatened by heavy ATV traffic.

Conscious of the effects of the slow recovery of the vegetation on the overall conditions in the village, the arboriginal community of Whapmagoostui wishes to restore the plant cover in the village. In order to provide guidelines to the local authorities, the main objective of our study was to evaluate the performance of three indigenous plant species (Leymus mollis, Lathyrus japonicus, and Trisetum spicatum) in response to mineral and organic fertilization. We conducted both greenhouse and field experiments to assess the impact of fertilization on emergence, survival, cover, and above- and belowground biomass.

## Material and Methods

#### STUDY AREA

The experiments were conducted in Whapmagoostui, the Cree community part of the Inuit–Cree village Kuujjuarapik– Whapmagoostui on the Hudson Bay coast at the mouth of the Grande Rivière de la Baleine in northern Québec, Canada  $(55^{\circ}17^{\prime}N, 77^{\circ}45^{\prime}W;$  Fig. 1). The village lies on deltaic sands left during the retreat of the Tyrell Sea or carried by the river. The region is experiencing one of the fastest isostatic rebounds, at a rate of 8 mm  $yr^{-1}$ . Climatic data provided by the Kuujjuarapik– Whapmagoostui weather station  $(55^{\circ}16^{\prime}N, 77^{\circ}45^{\prime}W; 10.4 \text{ m})$ above sea level) reveal an annual mean temperature of  $-4.4 \text{ }^{\circ}\text{C}$ for the period 1971–2000. Annual precipitation averages 648.5 mm, of which 37% falls as snow (Environnement Canada, 2007). The average frost free period is ca. 126 days. January is the coldest month ( $-23.4$  °C), while August is the warmest (11.4 °C).

The dune system adjacent to the village supports a high diversity of plant species. On the upper beach, Honkenya peploides forms small mounds called embryo dunes (Gagné and Houle, 2002). The foredune itself is dominated by Leymus mollis and, to a lesser extent, by Lathyrus japonicus. The fixed dunes, which represent the reference ecosystem for the village, are colonized by at least 27 vascular plant species including the ericaceous shrubs Vaccinium vitis-idaea and Empetrum hermaphroditum, 9 lichen species, and 8 moss and liverwort species (Desormeaux, 2005).

Soil supporting the fixed dune community can be described as regosol (unconsolidated material without stones and without distinct horizons) with a poorly developed organic layer  $(<5$  cm) often mixed with sand (Payette, 1973). In the village these soils were destroyed by the disturbances linked to human activities. As a result, most of the village area is covered with bare sand with no organic material and lower nutrient availability than the reference ecosystem (Table 1).

## STUDY SPECIES

We chose to focus on herbaceous species since most of the village was originally covered in herbaceous vegetation similar to the fixed dunes (Desormeaux, 2005). Houle and Babeux (1994b) also mentioned that the use of herbaceous species like Leymus mollis and Lathyrus japonicus should be considered. In previous restoration trials in Kuujjuarapik–Whapmagoostui, they investi-



FIGURE 1. Location of Kuujjuarapik–Whapmagoostui. The experiments were conducted in the Cree village of Whapmagoostui only.

gated the influence of fertilizing and mulching on the performance of woody species although they encountered serious vandalism problems (Houle and Babeux, 1994a).

We chose three herbaceous plant species based on their success in natural regeneration in nearby areas (Desormeaux, 2005), their availability as seed, and their aesthetics. Leymus mollis Trin. is a tall perennial grass (approximately 100 cm in height) that forms an extensive system of horizontal and vertical rhizomes that literally becomes the skeleton of the subarctic foredunes on the east coast of Hudson Bay. Its fruits are small, linear caryopses about 4–5 mm in length. It has an amphi-Atlantic distribution, being present in Iceland, Greenland, North America, and eastern Asia. Lathyrus japonicus Willd. is a medium-sized perennial plant (50–80 cm in height), also found on the foredune, that has the ability to fix atmospheric nitrogen via symbiotic relationships with soil bacteria (Rhizobium spp.). It has a circumpolar distribution and has been recorded in northwestern and eastern Canada, Greenland, Iceland, Britain, Denmark, Norway, Finland, eastern Siberia and Japan. Trisetum spicatum (L.) Richt., a short perennial grass growing to 30 cm, is a late successional species found mainly on older dunes in the area. It is found throughout the northern hemisphere.

Seeds of these species used in the following experiments were either harvested from the dune system nearby in August 2005 (L. mollis and T. spicatum) or bought from a commercial seed provider (L. japonicus). All seeds were stored in a cold chamber at  $4^{\circ}$ C for 8 months.

## Greenhouse Experiment

A greenhouse experiment was conducted at the Kuujjuarapik–Whapmagoostui Research Station of Centre d'études nordiques to evaluate the performance of L. mollis, L. japonicus, and T. spicatum to fertilizer addition. The three species were grown for 10 weeks in the sandy substrate from bare surfaces in the village with different levels of mineral (none, single, and double dose) and organic fertilizer (with or without). These six treatments  $(3 \times 2)$ were replicated five times in a fully factorial design for a total of 30 pots per species.

In the last week of May 2006, substrate was collected from different bare areas in the village. At each location, the topsoil (ca. 15 cm) was collected. Substrate was then sieved (2-mm mesh) to remove seeds and plant material. The following week, 100 filled

Chemical properties of the sandy substrate from the village used in both greenhouse and field experiment and of the marsh substrate used as an organic fertilizer.

| Soil variables                 | Bare surfaces<br>(village) | Fixed dunes<br>(reference) | Marsh<br>(organic fert.) |
|--------------------------------|----------------------------|----------------------------|--------------------------|
| $N(\%)$                        | $0.0078 \pm 0.00077$       | $0.037 \pm 0.0056$         | $0.38 \pm 0.23$          |
| $P$ (ppm)                      | $11 \pm 2.1$               | $36.64 \pm 7.69$           | $30 \pm 18$              |
| $K$ (ppm)                      | $13 \pm 4.8$               | $29.19 \pm 4.30$           | $78 \pm 44$              |
| $C(\%)$                        | $0.11 \pm 0.021$           | $0.70 \pm 0.13$            | $6.4 \pm 3.6$            |
| pH (CaCl <sub>2</sub> )        | $5.20 \pm 0.52$            | $3.83 \pm 0.07$            | $4.2 \pm 0.18$           |
| Ca (cmol(+) $kg^{-1}$ )        | $0.22 \pm 0.17$            | $0.44 \pm 0.11$            | $4.5 \pm 2.2$            |
| $Mg$ (cmol(+) $kg^{-1}$ )      | $0.023 \pm 0.0090$         | $0.29 \pm 0.058$           | $0.81 \pm 0.42$          |
| Na (cmol(+) $kg^{-1}$ )        | $0.032 \pm 0.0057$         | $0.046 \pm 0.0057$         | $0.25 \pm 0.12$          |
| K (cmol(+) $kg^{-1}$ )         | $0.050 \pm 0.014$          | $0.082 \pm 0.017$          | $0.18 \pm 0.10$          |
| Al $(cmol(+) \text{ kg}^{-1})$ | $0.029 \pm 0.011$          | $0.19 \pm 0.035$           | $0.82 \pm 0.50$          |
| Mn (cmol(+) $kg^{-1}$ )        | $0.0011 \pm 0.00018$       | $0.023 \pm 0.0067$         | $0.018 \pm 0.012$        |
| Fe (cmol(+) $kg^{-1}$ )        | $0.0053 \pm 0.0022$        | $0.021 \pm 0.0054$         | $0.069 \pm 0.044$        |

seeds (likely viable) of each species were sown at a depth of 2 cm in 1850 cm<sup>3</sup> pots filled with this sandy substrate.

For the mineral fertilization treatments we used a 20-20-20 NPK fertilizer (Plant-Prod company) that dissolves in water. The single dose treatment (4 g  $L^{-1}$ ) was based on the fabricant recommendation, while the double dose treatment (8 g  $L^{-1}$ ) was added to saturate nutrient availability. Mineral fertilizer treatments were administered every other week  $(250 \text{ mL pot}^{-1} \text{ wk}^{-1}; 100 \text{ mL})$ on the first and third days, 50 mL on the fifth). At the end of the experiment (after 10 weeks), pots assigned to the single and double dose treatments of mineral fertilization would have received 1 g and 2 g, respectively, of each of the main nutrients (N, P, K).

In order to provide a low-cost alternative to the use of mineral fertilizer, substrate from a small marsh dominated by Carex spp. just outside the village was used as an organic fertilizer. Samples were collected before the onset of the experiment and were sieved (2-mm mesh) to remove roots and debris. The organic fertilizer addition treatment was administered at the beginning of the experiment by replacing ca. 460 mL of the sand from the 1850 cm<sup>3</sup> pots with an equivalent volume of marsh substrate. Both substrates were then mixed together. In order to quantify the impact of this treatment on nutrient availability, 10 samples from the marsh were sent for chemical analyses. Results indicate that organic fertilization would have increased the total amount of N and organic matter per pot (ca. 2.8 g pot<sup>-1</sup> and 47.5 g pot<sup>-1</sup>), but would have had almost no impact on the amount of P and K (ca. 0.02 and 0.05 g  $pot^{-1}$ , respectively; Table 1). Moreover, the addition of marsh substrate would have resulted in an increase of the fraction of the sediment composed of finer particles (granulometry not shown), therefore increasing the water retention capacity of the substrate.

During the 10-week experiment, ambient day length was maintained at a minimum of 14 h and temperature fluctuated around 20  $\degree$ C, although it reached 30  $\degree$ C during a few very hot days. Pots were watered every other day with 100 mL of tap water. This volume (350 mL pot<sup>-1</sup> week<sup>-1</sup>) was calculated by multiplying the average weekly precipitation for June, July, and August  $(1.9 \text{ cm})$  with the pot area  $(176.7 \text{ cm}^2)$ . Seedling emergence was recorded once a week by counting the number of seedlings in each pot. At the end of the experiment, seedlings were carefully washed over a 1-mm mesh sieve to prevent any loss of root biomass. Afterwards, water used to wash the seedling was poured twice through the sieve to recuperate any biomass that would have been lost during the previous manipulations. Biomass was then sorted

into below- and aboveground parts. Tissues were dried at  $75 \text{ }^{\circ}\text{C}$ for 48 h and weighed.

## Field Experiment

Experimental plantations were conducted in exclosures (56  $m^2$ ; 8 m  $\times$  7 m) located in the courtyards of residents at different locations in the village. Exclosures were located on sandy substrate comparable to that used in the greenhouse experiment. Although their locations were suggested by the local authorities to minimize vandalism, two of the eight exclosures originally set up were vandalized and were therefore unusable. Each of the six remaining exclosures were divided into twenty  $1-m^2$  quadrats with a 50-cm buffer zone in between each quadrat to avoid fertilizer contamination and to allow pedestrian movement. Of these 20 quadrats, 8 were used for a second experiment not presented here.

The impact of organic and mineral fertilizer on the performance of the different species was evaluated in a  $2 \times 2$  factorial design for a total 12 plant species–treatment combinations. There were two levels of each type of fertilization (with and without). As in the greenhouse experiment, marsh substrate was used as organic fertilizer. Prior to seeding, a 2-cm-thick layer of marsh substrate was mixed into the superficial layer of sand (5 cm). For the mineral fertilization treatment we used a slow-release 14-14-14 NPK fertilizer (released over 70 days) to minimize manipulations throughout the experiment. In each  $1-m^2$  quadrat assigned to this treatment, 50 g of mineral fertilizer was added at the time of sowing (first week of July 2006). By the end of the experiment (13 months), quadrats assigned to the mineral fertilizer addition treatment would have received 7 g of each of the main nutrients (N, P, K).

In the first week of July 2006, seeds of *L. mollis* (12  $\text{g m}^{-2}$ ; ca. 1300 seeds), L. japonicus (10 g m<sup>-2</sup>; ca. 300 seeds), and T. spicatum (12 g m<sup>-2</sup>; ca. 50,000 seeds) were sown in each quadrat that had been prepared with or without organic or mineral fertilizer as described above. Seeds were then covered with ca. 1 cm of sand to minimize seed displacement by wind. Each  $1-m^2$  quadrat was watered once with 2.5 L of water in the last week of July 2006 during a dry period. Otherwise, water availability relied on natural precipitation (242.6 mm from 1 July to 30 September in 2006; 223.4 mm from 1 June to 15 August in 2007). In the second week of August 2007, vegetation cover was evaluated in a 50  $\times$  50 cm sub-quadrat within each 1-m<sup>2</sup> quadrat. We developed a method using a leaf area meter to analyze digital pictures. Digital pictures of each quadrat taken at a height of 1.2 m were transformed in Photoshop Elements 4.0 to isolate the pixels associated with the vegetation cover. We then used an AM300 Portable Leaf Area Meter (ADC BioScientific Ltd.) to determine the proportion of the image that was occupied by pixels associated with the vegetation cover. Also in August 2007 we sampled the aboveground biomass in a 100-cm<sup>2</sup> sub-quadrat located at the center of each quadrat. We used the small quadrat size in order to leave most of the vegetation cover for future monitoring. We did not sample belowground biomass in the field since it would have been too difficult without any breakage.

#### Statistical Analyses

All analyses were performed with the Statistical Package for Social Sciences (SPSS, v. 13.0 for Windows). Emergence of the different species throughout the greenhouse experiment was analyzed with the General Linear Model (GLM) Repeated Measures procedure due to temporal autocorrelation of the samples. A GLM multivariate procedure, which provides analysis

Repeated measures ANOVAs on the number of individuals of L. mollis, L. japonicus, and T. spicatum in response to mineral (MF) and organic (OF) fertilization treatment at different times during a 10-week fully factorial block design greenhouse experiment. The Huynh-Feldt adjusted p-values are presented; significant p-values are in bold.

| Species      | Factor     | df      | $\overline{MS}$ | F     | $\boldsymbol{p}$ |
|--------------|------------|---------|-----------------|-------|------------------|
| L. mollis    | Time       | 4.648   | 20,905.0        | 383.6 | $<$ 0.001 $\,$   |
|              | Time*Block | 18.590  | 50.4            | 0.9   | 0.553            |
|              | Time*MF    | 9.295   | 1531.8          | 28.1  | < 0.001          |
|              | Time*OF    | 4.648   | 131.6           | 2.4   | 0.082            |
|              | Time*MF*OF | 9.295   | 46.1            | 0.9   | 0.532            |
|              | Error      | 92.950  | 54.5            |       |                  |
| L. japonicus | Time       | 3.404   | 5708.2          | 138.1 | < 0.001          |
|              | Time*Block | 13.616  | 61.6            | 1.5   | 0.141            |
|              | Time*MF    | 6.808   | 841.2           | 20.4  | < 0.001          |
|              | Time*OF    | 3.404   | 226.2           | 5.5   | < 0.001          |
|              | Time*MF*OF | 6.808   | 33.2            | 0.8   | 0.536            |
|              | Error      | 68.078  | 41.3            |       |                  |
| T. spicatum  | Time       | 6.822   | 6032.8          | 198.2 | < 0.001          |
|              | Time*Block | 27.288  | 28.5            | 0.9   | 0.562            |
|              | Time*MF    | 13.644  | 501.1           | 16.5  | < 0.001          |
|              | Time*OF    | 6.822   | 255.4           | 8.4   | < 0.001          |
|              | Time*MF*OF | 13.644  | 201.2           | 6.6   | $<$ 0.001 $\,$   |
|              | Error      | 136.441 | 30.4            |       |                  |
|              |            |         |                 |       |                  |

of variance for multiple dependent variables by one or more factor variables, was performed on all species for the following variables: belowground and aboveground biomass at the end of the greenhouse experiment and percent cover and aboveground biomass at the end of the field experiment. Afterwards, GLM univariate analyses were conducted for each variable on individual species. Because the data were not normally distributed, belowground and aboveground biomass of L. mollis and L. japonicus and belowground biomass of T. spicatum were log-transformed, and aboveground biomass of T. spicatum for the greenhouse experiment was rank-transformed. Rank transformation was achieved by replacing each observation with its respective rank within a sample. LSD post-hoc tests were conducted to test for differences among the three levels of mineral fertilization if mineral fertilization had a significant effect in the greenhouse experiment, and for differences among all possible treatment combinations if there was a significant interaction between mineral and organic fertilization in both greenhouse and field experiments.

#### **Results**

#### GREENHOUSE EXPERIMENT

The emergence of the three species varied through time and as a function of the different treatments. Mineral fertilizer, either at a single or double dose, had a positive effect on L. mollis emergence (Table 2, Fig. 2). This impact was significant from week 3 to the end of the experiment (based on LSD test results, not shown). Organic fertilization had no effect on L. mollis emergence. In contrast, the emergence of L. japonicus was negatively affected by the addition of mineral fertilizer (Table 2, Fig. 2). As early as week 2, the emergence of L. japonicus in the non-fertilized pots was greater than in those that received the double dose of mineral fertilization. From week 6 until the end of the experiment, the three treatments of mineral fertilization differed significantly from each other. The emergence of L. japonicus was also negatively



FIGURE 2. Emergence of Leymus mollis, Lathyrus japonicus, and Trisetum spicatum in response to mineral and organic fertilizer (white symbols: no organic fertilization; black symbols: with organic fertilization; diamond: no mineral fertilization; triangle: single dose of mineral fertilization; square: double dose of mineral fertilization).

affected by the addition of organic fertilizer starting from week 2. The emergence response of T. spicatum to fertilizer addition was more complex: statistical analysis revealed an interaction between the two fertilization types (Table 2, Fig. 2). The negative impact of organic fertilization was aggravated when combined with a single or double dose of mineral fertilization.

The aboveground and belowground biomass of the three species varied as a function of the different treatments as revealed by the multivariate analysis (species  $\times$  mineral fertilization  $\times$ organic fertilization, Wilks' Lambda: 0.288,  $F_{[8,134]} = 14.452$ ,  $p <$ 0.001). For L. mollis, the production of both aboveground and belowground biomass resulted from an interaction between mineral and organic fertilization (Table 3, Fig. 3). Organic fertilization had a negative impact on aboveground and belowground biomass when combined with a double dose of mineral fertilization, while it had a positive impact when combined with the two other treatments. Mineral fertilization increased both belowground and aboveground biomass, particularly when applied at a single dose. Belowground and aboveground biomass of L. japonicus was reduced when either organic fertilization or a double dose of mineral fertilization was applied; the latter had a particularly strong effect (Table 3, Fig. 3). Both aboveground and belowground biomass of T. spicatum responded to an interaction between both fertilizer types (Table 3, Fig. 3). A single dose of mineral fertilization had a positive impact on belowground and aboveground biomass only when no organic fertilizer was applied. Moreover, organic fertilizer had a positive impact on belowground biomass only when no mineral fertilizer was added.

#### FIELD EXPERIMENT

According to the multivariate analyses, the cover and the aboveground biomass of the different species varied among the different exclosures (blocks, Wilks' Lambda: 0.598,  $F_{[10,108]}$  = 3.166,  $p = 0.001$ ), probably related to different sand burial during the growth season. Moreover, the intensity of the response to mineral fertilization varied from one species to the other (species \* MF, Wilks' Lambda: 0.410,  $F_{[4,108]} = 15.152, p < 0.001$ ; Fig. 4). However, the multivariate analysis revealed that the addition of

ANOVAs on the belowground and aboveground biomass of L. mollis, L. japonicus, and T. spicatum in response to mineral (MF) and organic (OF) fertilization treatment in a fully factorial block design greenhouse experiment. Significant p-values are in bold.

|                | Factor       | df             | $\overline{MS}$ | F      | $\boldsymbol{p}$ |
|----------------|--------------|----------------|-----------------|--------|------------------|
| L. mollis      |              |                |                 |        |                  |
| Belowground    | Block        | 4              | 0.0             | 0.255  | 0.010            |
| <b>Biomass</b> | МF           | $\overline{2}$ | 0.2             | 71.204 | < 0.001          |
|                | OF           | 1              | 0.0             | 4.522  | 0.118            |
|                | $MF*OF$      | $\overline{2}$ | 0.2             | 56.899 | < 0.001          |
|                | Error        | 20             | 0.0             |        |                  |
| Aboveground    | <b>Block</b> | 4              | 65.2            | 0.913  | 0.122            |
| <b>Biomass</b> | MF           | $\overline{2}$ | 2817.4          | 39.429 | < 0.001          |
|                | OF           | 1              | 681.6           | 9.539  | 0.094            |
|                | $MF*OF$      | $\overline{c}$ | 935.4           | 13.091 | 0.004            |
|                | Error        | 20             | 71.5            |        |                  |
| L. japonicus   |              |                |                 |        |                  |
| Belowground    | Block        | 4              | 0.0             | 1.276  | 0.312            |
| <b>Biomass</b> | MF           | $\overline{2}$ | 0.6             | 44.617 | < 0.001          |
|                | OF           | 1              | 0.2             | 12.400 | 0.002            |
|                | $MF*OF$      | $\overline{c}$ | 0.0             | 0.974  | 0.395            |
|                | Error        | 20             | 0.0             |        |                  |
| Aboveground    | <b>Block</b> | 4              | 0.0             | 1.368  | 0.280            |
| <b>Biomass</b> | MF           | $\overline{2}$ | 0.7             | 28.749 | < 0.001          |
|                | OF           | 1              | 0.1             | 4.662  | 0.043            |
|                | $MF*OF$      | $\overline{2}$ | 0.0             | 0.432  | 0.655            |
|                | Error        | 20             | 0.0             |        |                  |
| T. spicatum    |              |                |                 |        |                  |
| Belowground    | Block        | 4              | 0.0             | 0.255  | 0.903            |
| <b>Biomass</b> | MF           | $\overline{2}$ | 0.2             | 71.204 | < 0.001          |
|                | OF           | 1              | 0.0             | 4.522  | 0.046            |
|                | $MF*OF$      | $\overline{2}$ | 0.2             | 56.899 | < 0.001          |
|                | Error        | 20             | 0.0             |        |                  |
| Aboveground    | <b>Block</b> | 4              | 0.0             | 0.913  | 0.500            |
| <b>Biomass</b> | MF           | $\overline{2}$ | 0.7             | 39.429 | < 0.001          |
|                | OF           | 1              | 0.1             | 9.539  | 0.016            |
|                | $MF*OF$      | $\overline{2}$ | 0.0             | 13.091 | 0.001            |
|                | Error        | 20             | 0.0             |        |                  |

organic fertilizer was not significant overall (OF, Wilks' Lambda: 0.925,  $F_{[2,54]} = 2.181, p = 0.123$ .

Univariate analyses on individual species showed that mineral fertilization had a positive impact on both cover and aboveground biomass of all three species, although the impact was less evident for L. japonicus (Table 4, Fig. 4). Maximal cover was achieved by T. spicatum (ca. 34%), followed by L. mollis (ca. 23%) and L. japonicus (ca. 4%). However, L. mollis had greater aboveground biomass (ca. 6 g/100 cm<sup>2</sup>), followed by *T. spicatum* (ca. 3 g/100 cm<sup>2</sup>) and *L.* japonicus (ca. 1 g/100 cm<sup>2</sup>). Organic fertilization appeared to increase aboveground biomass for L. mollis when there was no mineral fertilization (see Table 4). However, this result should be interpreted carefully since it was not protected by the results of the multivariate analysis, i.e. the overall multivariate analysis did not reveal any significant effect of the organic fertilization.

## **Discussion**

## MINERAL FERTILIZATION

Overall, moderate mineral fertilization had a beneficial effect on the emergence and growth of L. mollis. The positive impact on emergence can be linked to an increase either in seed germination rate or in seedling emergence and survival. Although no experiment was conducted directly to identify the processes behind this result, overall seedling mortality was very low throughout the experiment, suggesting that nutrient addition might trigger L. mollis seed germination. These results corroborate studies on other grass species that showed the same tendencies : Leymus arenarius (Greipsson and Davy, 1997), Agropyron desertorum (Simons and Gross, 1985; Romo, 2005), Ammophila arenaria (van der Putten, 1990). Our greenhouse experiment results suggest that a single dose (4  $g L^{-1}$ , equivalent to the addition of 1 g each of N, P, and K per pot by the end of the experiment) is sufficient to improve the performance of this species. These results are in accordance with those of Houle (1997) and Gagné and Houle (2002), in which the addition of 720 mg and 56 mg per pot, respectively, were sufficient to have a positive impact on L. mollis biomass. However, individuals in our greenhouse study that were subjected to the double dose of mineral fertilization had lower root biomass than those subjected to the single dose. In the context of restoration of vegetation cover on sandy areas, where substrate stabilization is an important part of the process, treatments should aim to maximize the production of root biomass.

Lathyrus japonicus and T. spicatum emergence were both negatively affected by mineral fertilization in the greenhouse experiment, although the cover of both species increased with mineral fertilization in the field experiment. The negative impact in the greenhouse seems to be linked to both lower rates of seed germination and higher seedling mortality in substrates where nutrients were abundant. The negative impact of mineral fertilizer on both species biomass was particularly evident when both aboveground and belowground biomass were strongly reduced by a double dose of mineral fertilization. These results suggest that either species performs better when grown in nutritionally poor substrate, at least under controlled conditions. A few hypotheses can be formulated about the specific mechanisms responsible for such negative impacts of mineral fertilization. Bradshaw (1987) has suggested that high concentration of nitrogen might strengthen L. japonicus seed dormancy. Alternatively, nitrogen and phosphorus toxicity have previously been reported for some plant species (Griffin et al., 1995; Benton Jones, Jr., 1998, respectively). For example, excessive amounts of phosphorus may interfere with the uptake and utilization of zinc. Finally, high nutrient concentrations might also have been beneficial for the population growth of soil pathogens, resulting in increased damage to the plants.

In the light of such results, a more appropriate treatment to ensure the establishment of L. *japonicus* may be to inoculate seedlings with aerobic bacteria (Rhizobium sp.) and endomycorrhizal fungi. Renaut et al. (1986) showed an increase in L. latifolius performance when inoculated with such organisms while Staley and Wright (1991) showed a positive impact of Rhizobium inoculation. In our experiment, ''natural inoculation'' in the greenhouse experiment is believed to have been low since the substrate was taken from bare sand surfaces with likely low populations of endomycorrhiza.

#### ORGANIC FERTILIZATION

Overall, organic fertilization from the marsh substrate had neutral or negative impacts on seedling emergence and final biomass both in the greenhouse and in the village. For L. mollis emergence, these results can be linked to the increase in water retention capacity that can result from the addition of a finer substrate (McKendrick, 1997). Substrate thermofluctuation, which is an important trigger for  $L$ . arenarius germination



FIGURE 3. Belowground and aboveground biomass of Leymus mollis, Lathyrus japonicus, and Trisetum spicatum in response to mineral and organic fertilizer (mean + 1 SD; MF0: no mineral fertilization; MF1: single dose of mineral fertilization; MF2: double dose of mineral fertilization; OF0: no organic fertilization; OF1: with organic fertilization). Bars with similar lower-case letters show combination treatments that are not significantly different. Capital letters (L. japonicus) indicate levels of mineral fertilization that are not significantly different.

(Greipsson and Davy, 1994), is reduced by improved water retention capacity. Bond (1952) also reported that germination of L. arenarius was negatively affected when the water retention capacity of the substrate was increased by the addition of straw. For L. japonicus, the negative impacts of organic fertilization may be associated with the increase in nutrient availability combined with substrate acidification, as this species normally grows in pH from 5.8 to 7.2 (the substrate from the marsh had a pH of 4.2, Table 1). The impact of organic fertilization on the emergence of T. spicatum in the greenhouse was more complex (3-factor interactions). For an



FIGURE 4. Cover and aboveground biomass of Leymus mollis, Lathyrus japonicus, and Trisetum spicatum in response to mineral and organic fertilizer in the field experiment (mean + 1 SD; MF0: no mineral fertilization; MF1: single dose of mineral fertilization; OF0: no organic fertilization; OF1: with organic fertilization).

unknown reason, the combination of organic and mineral fertilization had a negative impact on T. spicatum performance.

The results we obtained do not corroborate the positive impacts of organic fertilization reported in other studies on organic matter and substrate stabilization (Elmarsdottir et al., 2003), legume survival during the first growing season (Densmore and Holmes, 1987), and the availability of N, P, K (Reid and Naeth, 2005). Organic fertilization is usually a natural alternative to mineral fertilization as it can help rebuild optimal soil conditions for the growth of various plant species (Johnson, 1987). However microbial mineralization of organic matter may be limited by low temperatures (Post et al., 1982) as well as by nutrient deficiency (Weintraub and Schimel, 2003; Schimel et al., 2004). In addition to increasing nutrient concentration, organic fertilization can increase cationic exchange capacity, resulting in a decrease in nutrient leaching. The negative effects we observed may be due to our source of organic fertilization, a marsh substrate. Other studies used manure (Elmarsdottir et al., 2003), top soil (Densmore and Holmes, 1987), or paper mill waste (Reid and Naeth, 2005); these organic materials may be higher in nutrients, may be less acidic, and may have less impact on water retention capacity, although more study is needed.

## **CONCLUSION**

Our study clearly showed that indigenous species can be used in assisted revegetation trials in subarctic environments. The three species used were able to get established in a natural environment during the first growing season, to survive the winter season, and to grow rapidly during the second growing season. While only the L. mollis performance was enhanced by mineral fertilization in the greenhouse experiment, the addition of mineral fertilizer in the field had a positive impact on all species (biomass and cover). Organic fertilization using substrate from the neighboring marsh ecosystem, on the other hand, did not enhance the

ANOVAs on the percentage cover and aboveground biomass of L. mollis, L. japonicus, and T. spicatum in response to mineral (MF) and organic (OF) fertilization treatment in a fully factorial block design field experiment. Significant p-values are in bold.

|                | Factor       | df             | $\overline{MS}$ | F       | $\boldsymbol{p}$ |
|----------------|--------------|----------------|-----------------|---------|------------------|
| L. mollis      |              |                |                 |         |                  |
| Cover          | Block        | 5              | 162.4           | 3.152   | 0.038            |
|                | MF           | 1              | 2245.6          | 43.578  | < 0.001          |
|                | OF           | 1              | 22.4            | 0.435   | 0.520            |
|                | $MF*OF$      | $\overline{c}$ | 0.8             | 0.016   | 0.900            |
|                | Error        | 15             | 51.5            |         |                  |
| Aboveground    | Block        | 5              | 9.7             | 7.640   | < 0.001          |
| <b>Biomass</b> | MF           | $\mathbf{1}$   | 141.8           | 111.627 | < 0.001          |
|                | OF           | $\mathbf{1}$   | 7.9             | 6.252   | $0.024*$         |
|                | $MF*OF$      | $\overline{c}$ | 0.1             | 0.050   | 0.827            |
|                | Error        | 15             | 1.3             |         |                  |
| L. japonicus   |              |                |                 |         |                  |
| Cover          | Block        | 5              | 5.1             | 1.069   | 0.416            |
|                | MF           | 1              | 31.6            | 6.561   | 0.022            |
|                | OF           | $\mathbf{1}$   | 6.4             | 1.329   | 0.267            |
|                | $MF*OF$      | $\overline{2}$ | 3.1             | 0.640   | 0.436            |
|                | Error        | 15             | 4.8             |         |                  |
| Aboveground    | Block        | 5              | 0.304           | 1.473   | 0.256            |
| <b>Biomass</b> | MF           | 1              | 1.7             | 8.200   | 0.012            |
|                | OF           | $\mathbf{1}$   | 0.1             | 0.700   | 0.432            |
|                | $MF*OF$      | $\overline{2}$ | 0.1             | 0.386   | 0.544            |
|                | Error        | 15             | 71.5            |         |                  |
| T. spicatum    |              |                |                 |         |                  |
| Cover          | Block        | 5              | 409.7           | 2.401   | 0.086            |
|                | MF           | 1              | 4038.3          | 23.671  | < 0.001          |
|                | OF           | $\mathbf{1}$   | 29.4            | 0.172   | 0.684            |
|                | $MF*OF$      | $\overline{c}$ | 115.0           | 0.674   | 0.425            |
|                | Error        | 15             | 170.6           |         |                  |
| Aboveground    | <b>Block</b> | 5              | 5.5             | 4.426   | 0.011            |
| <b>Biomass</b> | MF           | 1              | 42.1            | 33.945  | < 0.001          |
|                | OF           | 1              | 0.0             | 0.003   | 0.956            |
|                | $MF*OF$      | $\overline{2}$ | 0.0             | 0.035   | 0.854            |
|                | Error        | 15             | 1.2             |         |                  |

\* Not protected by the multivariate analysis.

performance of the different species. The low cover and aboveground biomass of L. japonicus after 13 months in the field experiment suggest that we need a better understanding of its ecology before using it in assisted revegetation trials. For example, the inoculation of young L. japonicus seedlings with vesiculararbuscular mycorrhizal fungi or aerobic bacteria of the Rhizobium genus, which were previously shown to increase the performance of L. latifolius (Renaut et al., 1986), might be a more appropriate treatment for this species.

Our results suggest that the sowing of L. mollis or T. spicatum seeds, combined with a single dose of mineral fertilizer, would provide a low-cost assisted revegetation treatment. We believe that the use of L. *mollis* would provide the best chance of success; however, *T. spicatum* may be more aesthetically pleasing to the village residents. Before any large-scale assisted revegetation project is undertaken with any of these species, actions must be taken by the local authorities to limit the negative impact of the heavy ATV traffic prevailing in the village. Without a drastic reduction of the use of ATVs off the main roads, assisted revegetation projects are condemned to fail.

The authors would like to thank Ian Boucher, Françoise Cardou, Patrick Dick, Geneviève Dufour Tremblay, Julie Faure-Lacroix, Steve Inish, Peter Natashequan, Pascale Ropars, and Marie-Pascale Villeneuve Simard for their help in the field and in the laboratory. They would also like to thank the Cree Regional Authority (Ginette Lajoie and Cameron MacLean), the Whapmagoostui Band Council and its representative (Robert Wynne), and the Centre d'études nordiques. This project was financially supported by the Northern Ecosystems Initiative and the Natural Sciences and Engineering Research Council of Canada (NSERC) via the Northern Research Chair on Disturbance Ecology.

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MS accepted April 2009