Initial fertilization of *Betula pubescens* in Iceland did not affect ectomycorrhizal colonization but improved growth

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ABSTRACT

Different doses of nitrogen (N) and phosphorus (P) were applied in a factorial design experiment to seedlings planted in nutrient poor and nutrient rich Andisols in Iceland to determine the effect of initial fertilization on ectomycorrhizal colonization, survival, growth, and nutrient status of downy birch (*Betula pubescens*) seedlings. One year after planting the highest NP fertilizer dosage reduced ectomycorrhizal colonization at the nutrient poor site significantly, while at the nutritional rich site the N and P fertilization had no significant effect. Three years after planting the difference in ectomycorrhizal colonization between the unfertilized and fertilized seedlings had vanished on both sites. At that time the average colonization at the rich and the poor site was 60% and 79%, respectively. No clear effect on the composition of ectomycorrhizal morphotypes was found by the various NP combinations. The initial fertilization increased the dry weight of birch on both soil types significantly. One and three years after planting the increase was up to 3.6 and 14 times, respectively. On the rich site, the largest dosages of NP had no additional effect over that of smaller NP dosages, while at the nutrient poor site the larger the dosages, the more the seedlings grew. Fertilization increased the foliar concentrations of N and P 3.6 and 7.7 times, respectively. NP fertilization during establishment of downy birch on Andisols in Iceland is recommended, as site-adapted moderate dosages had a positive effect on the growth and nutrient status and no adverse effect on the ectomycorrhizal development three years after planting.

Keywords: afforestation, containerized seedlings, forest fertilization, seedling nutrition, survival

YFIRLIT

Áburðargjöf við gróðursetningu á birki jók vöxt en hafði ekki áhrif á þróun svepprótar

Árið 1998 var gerð áburðartilraun á birki (*Betula pubescens*) á tveimur stöðum á Suðurlandi. Markmið tilraunarinnar var að athuga áhrif mismunandi skammta af nitri (N) og fosfór (P) á lifun, vöxt, næringarástand og svepprótarmyndun á birki, annarsvegar á frjósömu landi (Kollabæ) og hins vegar á rýru landi (Markarfljótsaurum). Borið var á plöntur einu sinni við gróðursetningu í júní 1998 og úttektir gerðar næstu þrjú árin þar á eftir. Einu ári eftir gróðursetningu hafði stærsti NP skammturinn marktæk neikvæð áhrif á myndun svepprótar á rótum birkisins á rýra landinu. Þremur árum eftir gróðursetningu voru áhrif áburðargjafanna á svepprótamyndun horfin og var magn svepppróta á rótarendum svipað hjá birki í öllum áburðarliðum. Meðaltíðni svepppróta á rótarendum var þá 60% á frjósama landinu og 79% á því rýra. Enginn munur var á samsetningu útlitsgerða svepppróta milli áburðarliðanna. Áburðargjöf hafði jákvæð áhrif á vöxt og var þurrvigt róta og yfirvaxtar birkis allt að 3,6 og 14 sinnum meiri í bestu áburðarmeðferðinni en hjá viðmiðunarplöntum, einu og þremur árum eftir gróðursetningu. Á frjósömu mólendi juku stærstu NP skammtarnir vöxt lítið samanborið við smærri NP skammta, en á rýrum malaraurum uxu plöntur því meira því stærri sem NP skammtarnir voru. Áburðargjöfin jók styrk næringarefna í birkilaufi haustið eftir gróðursetningu um 3,6 (N) og 7,7 (P). Út frá niðurstöðum tilraunarinnar er óhætt að mæla með áburðargjöf á birki við gróðursetningu, enda jók hún vöxt og bætti næringarástand án þess að hafa neikvæð áhrif á þróun svepppróta á plöntunum þremur árum eftir gróðursetningu.

INTRODUCTION

Downy birch (*Betula pubescens* Ehrh.) is a native tree species that presently covers about 1.2% of the total surface area of Iceland (Sigurdsson & Snorrason 2000). It is generally believed that in the late 9th century when Iceland was settled about 25-30% of the surface area of the country was covered by birch woodlands and forests (Blöndal 1993). The subsequent large scale deforestation has been explained by the combined effect of heavy livestock grazing, felling of trees for charcoal and firewood, forest clearing for grazing land, frequent volcanic eruptions and the cooler climate during the Middle Ages (Bjarnason 1942). Today, downy birch is one of the most planted tree species in Iceland. In 2004 more than 1.5 million seedlings of downy birch were planted on an area of 500-600 ha, mainly in connection with afforestation of treeless nutrient deficient land (Gunnarsson 2005). A new project, where birch will be used for land reclamation and erosion prevention on an area of about 60,000 ha in southern Iceland, has recently been initiated (Aradóttir 2007).

Success of afforestation in Iceland has in many cases been poor (Oskarsson & Ottosson 1990). Often the reason has been low survival and growth during the establishment phase, but also partly due to specific site conditions and management practices (Oskarsson & Ottosson 1990, Aradóttir & Grétarsdóttir 1995). Deficiency of nitrogen and phosphorus has been identified as a major limiting factor for growth of newly planted tree seedlings in Iceland (Oskarsson & Sigurgeirsson 2001). Vitalization fertilization with nitrogen and phosphorus at the time of planting has given positive growth effects on newly planted tree seedlings (Guðmundsson 1995, Oskarsson et al. 2006).

Many studies have shown that N and P fertilization has negative effects on the formation of ectomycorrhiza (see reviews by Wallander 1995, Wallenda & Kottke 1998, Allen et al. 2003, Treseder 2004). However, the results depend on soil type and soil nutrient status (Treseder & Vitousek 2001), and recently, nitrogen additions have been found to increase ectomycorrhiza in arctic nitrogen deficient ecosystems (Clemmensen et al. 2006), a finding that contradicts earlier results. Many studies have looked at the short-term effect of N or P additions on ectomycorrhiza (e.g. Treseder & Allen 2002, Dixon et al. 1981, Newton & Pigott 1991a). However, fewer studies have dealt with the long-term effect of a single addition of nitrogen and phosphorus on ectomycorrhizal development (e.g. Tétreault et al. 1978). As a single application of NP fertilizer is a common practice during afforestation in Iceland, this is an important research subject.

This paper presents a three year study of downy birch that received various dosages of N and P at the time of planting. This was tested on two contrasting soil types in South Iceland, a gravel soil extremely low in nutrients and relatively fertile abandoned grazing land. The main goals were to quantify the effects of initial fertilization of birch seedlings with different dosages and combinations of easily soluble nitrogen and phosphorus on the development of ectomycorrhizal colonization and seedling survival and growth. Furthermore the foliar nutrient concentration was measured.

MATERIALS AND METHODS

Site description

The study was located at two sites in southern Iceland, Markarfljótsaurar (63°40.158'N and 20°00.554'V, 60 m a.s.l.) and Kollabær (63°44.758'N and 20°03.205'V, 135 m a.s.l.). These sites will be identified as the nutrient poor M-site and the rich K-site, respectively. The poor M-site is located on a flat and barren outwash plain of the River Markarfljót, near the south coast. The river flooded regularly over the area until the building of revetments in the 1930's. It is sparsely vegetated, primarily by low shrubs (*Empetrum nigrum* L. and *Thymus praecox ssp. arcticus* (Dur.) Ronn. and grass species (*Festuca* sp. and *Agrostis* sp.). The soil at the poor M-site is mostly a mixture of basaltic sand and gravel and the nutritional status of such soils is generally believed to be poor (Johannesson 1960). The rich K-site is located on a hilltop 8 km NW from Markarfljótsaurar. It is fully vegetated, primarily by tussocks covered with thick moss (*Rhacomitrium* sp.) and with some grass cover in between. The soil at the K-site has a silt-loam texture and is at least 1 m thick due to the long lasting deposition of aeolian materials originating from nearby erosion areas. Ash and pumice layers, originating from volcanoes in the vicinity, are visible at various depths in the soil profile. The soil type of the K-site is moderately fertile compared to Icelandic conditions (Johannesson 1960).

Furrows were ploughed in the soil surface in the spring of 1998 at both sites using an agricultural plough. The plough formed furrows 10-20 cm deep and 30-40 cm wide, and mounded the spoil into a ridge about 20 cm high. The aim of this soil preparation was to create a favourable microclimate for seedlings by improving soil temperature and moisture, to reduce competition from other vegetation, and to create a shelter. This is a common site preparation method used in afforestation in Iceland, especially on grassland.

The experiment was initiated on 26-27 June 1998. One-year-old containerized seedlings of

Table 1. Fertilizer treatments and amounts in grams pr. seedling. The ´x´ indicates from which treatments seedlings were excavated in 1999 and 2000.

Treatment	N	P	33% NH_4NO_3	Triple phosphate	Sampled in 1999	Sampled in 2000
N0P0	0	0	0	0	x	x
N0P15	0	6.3	0.0	33.3	x	x
N0.5P5	0.5	2.1	1.5	11.1		
N0.5P10	0.5	4.2	1.5	22.2		
N0.5P15	0.5	6.3	1.5	33.3		x
N0.5P20	0.5	8.4	1.5	44.4		
N1P0	1	0.0	3.0	0.0	x	x
N1P5	1	2.1	3.0	11.1		
N1P15	1	6.3	3.0	33.3	x	x
N1P20	1	8.4	3.0	44.4		
N1P25	1	10.6	3.0	55.6		
N2P10	2	4.2	6.1	22.2		
N2P15	2	6.3	6.1	33.3		x
N2P20	2	8.4	6.1	44.4		
N4P5	4	2.1	12.1	11.1		
N4P15	4	6.3	12.1	33.3		x
N8P15	8	6.3	24.2	33.3		x
N8P25	8	10.6	24.2	55.6	x	

downy birch (*Betula pubescens* Ehrh. prov. Embla), with root volume of 100 cm^3 were used. Planting was performed manually with a planting tube (Pottiputki, Lannen Corp., Iso-Vimma, Finland) at a spacing of 1x2 m: hence the planting density was 5,000 seedlings ha^{-1}.

Fertilizer was applied at the time of planting and was scattered by hand over a 15-20 cm circumference around the seedlings. Eighteen combinations of nitrogen and phosphorus were applied as easily soluble nitrogen (33% N as NH_4NO_3) and phosphorus (triple phosphate: 19% P as $Ca(H_2PO_4)_2$) (Table 1). In afforestation in Iceland the usual dosages for small tree seedlings is 1 g N and 15 g P. In the present study the combinations selected ranged from zero N and P up to two and eight times the normal dosages of P and N, respectively (Table 1). All treatments were randomized within each of the four blocks on the two sites. Each plot within the blocks contained 20 seedlings, resulting in a total of 2,880 birch seedlings.

The weather conditions during the first year of the experiment were favourable for tree growth both as regards temperature and precipitation. No occurrences of damaging frost in the growing season were measured at a nearby meteorological station (Hella, 20 and 26 km from the sites) during the experimental period, 1998-2000 (pers. comm. Icelandic Meteorological Office 2004). The mean annual temperature during the years 1998-2000 was 3.9 °C. The mean temperature of the four warmest months (May-September) and the coldest month (January) during 1998-2000 was 9.6 and –1.0 °C, respectively. The effective temperature sum (degree-days, > +5 °C) during the years 1998, 1999 and 2000 was 773, 756 and 1,182 d.d., respectively. The precipitation during the growing season (May-September) in 1998-2000 was on average 508 mm.

One soil profile was dug on each experimental site. At the K-site samples were taken at three depths: 0-5, 6-15, and 30-45 cm, but at the poor M-site the soil was uniform gravel with no recognizable soil horizons, and therefore samples were collected only from one depth, 0-15 cm. The soil samples were oven-dried at 70°C for 24 hours and crushed gently to pass a 2 mm sieve before analyses. At the poor M-site about half of the sample was coarser than 2 mm and was therefore not used for analyses. The coarseness was however accounted for when the data were analyzed. Carbon was determined by dry combustion at 1,050°C using a Leco CR-12 C analyser. Nitrogen was determined by the Kjeldahl method. Soil pH was determined in a 1:10 soil:water suspension using a combination calomel electrode. Phosphorus soluble in 0.5 M $NaHCO_3$ was determined as described by Olsen et al. (1954). Exchangeable potassium was extracted by 1.25% HAc and determined by flame emission spectrometry.

Sampling and processing of data

The experiments were assessed at the end of the growing season during each of the first three years after planting. All seedlings were assessed for survival and frost heaving. The frost heaving of seedlings was measured as lifting of the root collar surface in cm compared to the surrounding soil surface. A random subset of eight seedlings from each treatment plot was measured each year for total height in spring and autumn and annual shoot elongation.

Random subsets of 4 seedlings from each of the selected treatments were excavated from every block at each site on two occasions, on 20 May 1999 and 25 August 2000. In 1999 the treatments that were showing the most contrasting growth responses were sampled. These were: N0P0, N1P0, N0P15, N1P15 and N8P25 (Table 1). The year after, only the treatments with increasing N dosages and fixed P dosage were excavated. These were: N0P0, N1P0, N0P15, N0.5P15, N1P15, N2P15, N4P15 and N8P15 (Table 1). The reason for this choice was that variation in growth in the second and third years was mainly due to variation in N dosage.

The upper part of each seedling, branches, leaves and stem, was separated from the root. Thereafter soil was carefully washed off the roots. The upper part and the roots were then oven-dried at 70°C for 72 h and the dry weight of each part was then determined.

Three root samples were cut off each of the harvested seedlings just after sampling. The samples were taken from a) the top of the root system, b) the middle of the root system, and c) the bottom of the root system. The root samples were stored in 13:5:200 formalin:acetic-acid:50%-ethyl alcohol liquid until further analysis. The ectomycorrhizal root tips were categorized into different morphotypes, M1-M13, on the basis of morphology, colour, characteristics of the surface of the hyphal mantle, and planar views of different mantle layers, using methods described by Agerer (1987-1998). Initial categorization and ectomycorrhizal colonization were quantified by counting the total number of root tips, at least 100, and the total number of ectomycorrhizal fine root tips and obtaining the percentage of colonization. Counting of root tips was carried out using a binocular dissecting microscope at a 10±30 magnification.

Foliage for chemical analyses was systematically sampled on 28 August 1998 from 5-15 randomly selected seedlings from the N0P0, N1P0, N0P15, N1P15, N4P5 and N8P25 treatments. The foliar samples were dried (70°C, 48 h), milled and analysed for ash content and N (Kjeldahl method). Other macro- and micro-nutrients (P, K, Ca, S, Mg, Mn, Fe, Zn, Cu and B) were determined with an Inductive Coupled Plasma (ICP-AES) analyser after being dissolved in concentrated HNO_3 in a microwave oven.

The statistical analyses were done separately for each of the two sites. Prior to analyses, the plot means were calculated for seedling dry weight, foliar nutrient content and ectomycorrhizal colonization. The plot means were used to perform the statistical tests in the general linear models (GLM) of the SAS system for Windows, release 8.02 (SAS Institute Inc., Cary, N.C.), for randomized block design. A Kolmorogrov-Smirnov test was used for testing the normal distribution of the data. The test was significant ($p>0.05$) for growth data and ectomycorrhizal colonization. Growth data were transformed by logarithm and data for ectomycorrhizal colonization were transformed by arcsin(sqrt(x)) before analysis of variance. Duncan's multiple range test was used to test for pairwise differences ($p>0.05$) between the treatment means. No statistical analyses were done on the ectomycorrhizal morphotypes, as the data were not normally distributed.

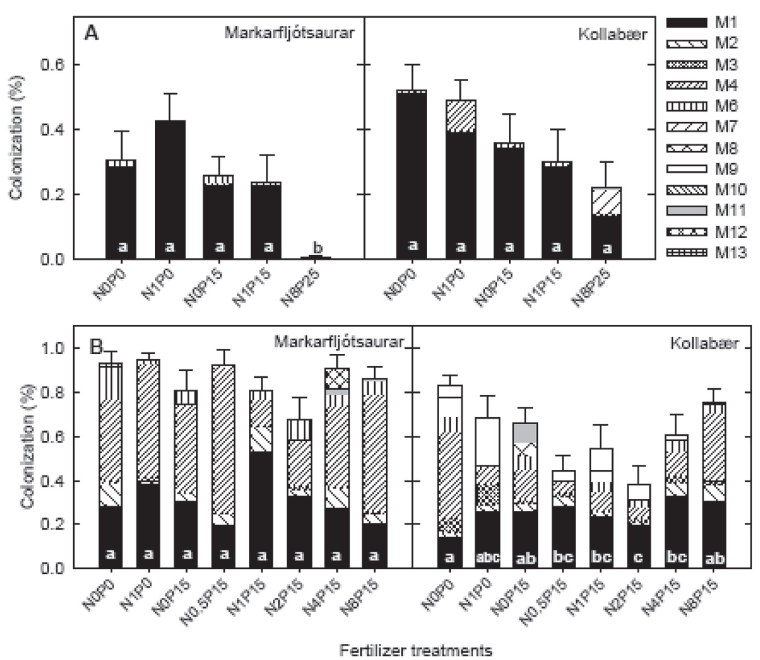

Figure 1. The proportional colonization of root tips by ectomycorrhiza on *Betula pubescens* seedlings and the different morphotypes (M1-M13) found (A) one and (B) three years after planting and fertilization at two sites. The letters within the column show a non-significant difference ($p < 0.05$) by Duncan's test on the total dry weight. The bars show standard error of means.

RESULTS

Ectomycorrhizal colonization

After one growing season the average ectomycorrhizal colonization of birch roots was higher at the nutritional rich K-site compared to the poor M-site (39% and 23%) (Figure 1). At the rich K-site there was no significant (p=0.15) treatment effect, but at the poor M-site this effect was significant (p=0.02). At the poor M-site the N1P0 treatment had on average the highest colonization (41%); however only the N8P25 treatment varied significantly from the other treatments with the lowest colonization, 0.3% (Figure 1A). In total six morphotypes were present after the first year, but the M1 morphotype was the dominating one on both sites. In some treatments this was the only morphotype (Figure 1A).

Three years after planting the ectomycorrhizal colonization was higher at the poor M-site, or 80% compared to 60% at the K-site. There was no significant (p=0.13) difference in colonization between fertilizer treatments at the poor M-site, but at the rich K-site the difference was significant (p=0.02). At that site the control seedlings had the highest colonization, 84%, along with N8P15, N0P15 and N1P0. The treatments N0.5P15 and N4P15 had the lowest colonization, ranging from 40 to 50% (Figure 1B). In total 12 morphotypes were present after the third year, and no single one had absolute dominance although M1 and M4 were the most common (Figure 1B).

Survival and frost heaving

The survival of seedlings was high in the experiment, with an overall survival after three years of 99.5% at the M-site and 98.0% at the K-site. Frost heaving was negligible in the trial, and only a few seedlings at the K-site were lifted by a few cm by frost action in soil. No significant treatment effects on survival or frost heaving were found (data not shown).

The annual shoot elongation and dry weight

At the poor M-site the overall average annual shoot elongation was 14.7, 7.8 and 2.4 cm in the first, second and third years, respectively. There was an overall significant effect (p<0.005) from the initial fertilization in all years. The treatments that gave the highest growth at the poor M-site in 1998 were those containing 2-8 g of N. In 1999 and 2000 the

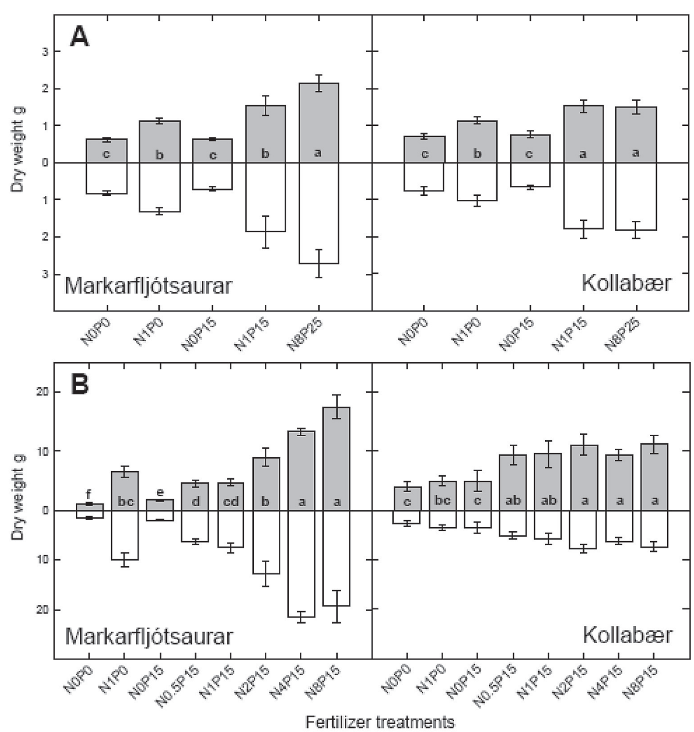

Figure 2. The dry weight of leaves, stem and branches (gray) and roots (open column) of *Betula pubescens* seedlings at the two sites (A) one and (B) three years after planting. The letters within the column show a non-significant difference (p < 0.05) by Duncan's test on the total dry weight. The bars show standard error of means.

N8P25 treatment (highest N and P) gave the highest annual shoot elongation (data not shown). The annual growth increments did not show a significant difference when various P dosages with fixed N were applied (data not shown). At the rich K-site the overall average annual shoot elongation was 16.0, 9.6 and 7.2 cm in the first, second and third years, respectively. There was a significant ($p<0.001$) overall fertilization effect on shoot elongation during the first two years, but in the third year the effect was no longer significant ($p<0.43$; data not shown).

One year after planting, the overall fertilization effect on the dry weight was significant ($p<0.001$) at both sites. That year, the seedlings receiving the N8P25 initial dose were at both sites the largest ones (Figure 2A).

Table 2. The nutrient concentration and nutrient to N ratios of *Betula pubescens* leaves at Markarfljótsaurar and Kollabær. Samples were collected two months after planting and fertilization on 28 August 1998. Means within columns followed by the same letter are not significantly different ($p<0.05$) by Duncan's test. '*' indicates a non-significant treatment effect ($p>0.05$).

		mg g^{-1}												mg kg^{-1}									
Treatment		N		P		K		Ca		S		Mg		Mn		Fe		Zn		Cu		B	
Markarfljótsaurar																							
N0P0	*(a)*	9.0	a	1.2	a	6.5	a	4.9	a	0.7	a	3.0	*	380	a	1020	*	82	ad	8	a	12	a
N0P15	Dry mass	10.1	a	6.5	c	7.5	ab	7.1	bc	0.9	b	4.0		529	b	929		103	ab	2	b	20	b
N1P0		26.0	b	1.6	a	6.7	ab	5.7	ab	1.2	c	3.3		378	a	1436		133	bc	7	a	9	d
N1P15		26.7	b	9.2	d	10.0	c	8.3	cd	1.6	d	3.6		372	a	831		148	c	6	a	30	c
N4P5		32.0	c	3.4	b	7.8	b	7.2	bc	1.8	d	3.2		309	a	769		58	de	6	a	14	a
N8P25		32.8	c	5.1	c	7.4	ab	9.6	d	2.1	e	3.7		315	a	885		36	e	6	a	26	bc
N0P0	*(b)*	-		13.9	ab	72.6	a	55.0	a	7.4	a	34.1	a	4.28	a	11.55	a	0.92	a	0.09	a	0.14	a
N0P15	N-ratio	-		63.6	c	74.3	a	69.9	b	9.0	b	39.6	a	5.24	a	9.35	a	1.02	a	0.02	b	0.20	b
N1P0		-		6.3	e	26.0	c	22.0	d	4.5	e	12.6	b	1.46	b	5.74	b	0.51	b	0.03	b	0.03	e
N1P15		-		34.5	d	37.5	b	31.0	c	6.1	cd	13.6	b	1.40	b	3.13	bc	0.56	b	0.02	b	0.11	a
N4P5		-		10.7	b	24.6	c	22.5	d	5.5	d	10.1	c	0.98	c	2.45	c	0.19	c	0.02	b	0.05	d
N8P25		-		15.4	a	22.6	c	29.3	c	6.3	c	11.3	bc	0.96	c	2.75	bc	0.11	d	0.02	b	0.08	c
Kollabær																							
N0P0	*(a)*	11.9	a	1.3	ab	6.1	a	5.9	*	0.8	a	3.3	*	341	a	990	*	93	*	5	*	16	*
N0P15	Dry mass	15.7	a	3.7	c	7.4	bc	6.4		1.0	b	3.2		297	ab	1006		151		7		18	
N1P0		29.3	bc	0.9	a	6.4	ab	5.8		1.3	c	3.4		280	ab	1135		118		5		19	
N1P15		26.2	b	2.9	a	8.1	c	6.5		1.6	d	3.4		246	b	1145		162		10		23	
N4P5		34.3	c	1.6	b	5.8	a	5.7		1.6	d	3.1		295	ab	1197		114		7		18	
N8P25		31.2	bc	3.3	a	6.5	ab	6.1		2.1	e	3.4		222	b	833		73		7		21	
N0P0	*(b)*	-		10.9	a	52.0	a	49.8	a	6.3	a	28.1	a	2.88	a	8.22	*	0.78	a	0.04	*	0.14	a
N0P15	N-ratio	-		21.8	b	45.2	a	39.7	b	6.2	a	19.4	b	1.82	b	6.24		0.90	a	0.04		0.11	ab
N1P0		-		3.0	c	22.1	c	20.0	cd	4.5	b	11.7	c	0.96	c	3.98		0.41	bc	0.02		0.06	cd
N1P15		-		10.9	a	31.4	b	25.2	c	6.1	a	13.5	c	0.93	c	4.55		0.62	ab	0.04		0.09	bc
N4P5		-		4.9	c	17.2	cd	17.0	d	4.6	b	9.0	d	0.87	c	3.92		0.33	cd	0.02		0.05	d
N8P25		-		10.2	a	20.7	d	19.8	cd	6.7	a	10.9	cd	0.71	d	2.80		0.23	d	0.02		0.07	cd
Target [1]		-		10		65		6		9		8		0.4		0.7		0.03		0.03		0.2	

[1] The target values show the relative levels of nutrients (N=100) that may be considered to be suitable for optimum nutrition of birch seedlings in culture solutions (Ericsson & Ingestad 1988, Ingestad 1971, Ingestad 1979).

The N8P25 seedlings at the poor M-site had a 48% higher dry weight than seedlings receiving the same treatment at the rich K-site. At the K-site there was no significant difference in dry weight among the N1P15 and N8P25 treatments (Figure 2A). The control seedlings (no initial fertilization) and seedlings receiving N0P15 had the lowest dry weight at both sites, around 1.4 g. Seedlings that received the lowest N dose (N1P0) had 57% and 71% higher dry weight than the control seedlings after one year at the rich K-site and the poor M-site, respectively (Figure 2A).

The initial fertilization also had a highly significant effect on the dry weight of seedlings three years after planting ($p<0.001$). At the rich K-site all treatments with both N and P additions (N2P15, N8P15, N4P15, N0.5P15 and N1P15) gave increased dry weight when compared to control seedlings. However, according to Duncan's multiple range test, there was no significant ($p>0.05$) difference among those treatments (Figure1B). At the poor M-site the highest N inputs (N8P15 and N4P15) gave the highest dry weight. Three years after the initial fertilization the largest plants at the poor M-site (N8P15) had 95% more dry weight than the largest plants at the K-site (N2P15). However, the control seedlings (N0P0) that had the lowest dry weight at both sites were 2.6 g/seedling at the poor M-site compared to 6.7 g/seedling at the rich K-site (Figure 2B). That is, the initial fertilization led to stronger growth response and larger birch plants at the poor M-site, but without the initial input the total birch seedling dry weight was only 40% of that at the rich K-site (Figure1B).

Foliar nutrient concentration

Fertilization treatments significantly affected the foliar nutrient concentration of birch seedlings two months after planting. The foliar nitrogen concentration was lowest in control seedlings 9.0 mg N g^{-1} at the poor M-site and 11.9 mg N g^{-1} at the rich K-site. At the poor M-site the N concentration increased with the N dosage (Table 2). The average concentration of P, K, Ca, and Mn was higher at the poor M-site, but the average N and Zn concentration was higher at the rich K-site (Table 2).

When P was lacking in the fertilizer treatment (N1P0) or when the level of P was relatively low (N4P5) the N/P ratio was significantly lower ($p<0.05$) than the N/P ratios of other treatments (Table 2). The ratio of Ca/N, Mg/N, Mn/N, Fe/N and Zn/N was generally higher than the target values shown to maintain optimum growth; however the N ratios were lower than the target ratios for B/N and K/N, especially when N was applied (Table 2).

Soil description

The organic soil carbon and N concentration at the M-site was low (1 g C kg-1 and 0.1 g N kg^{-1}), while at the K-site the concentration was much higher (108 g C kg^{-1} and 6.4 g N kg^{-1}). At the K-site both N and C were highest in the surface layer and decreased with depth (Table 3). Soil pH in the surface layer was 5.9 at the M-site, but 5.4 at the K-site and increased with depth. The concentration of P, however, was slightly higher at the M-site than at the K-site (Table 3).

DISCUSSION

Ectomycorrhizal colonization

The present study was aimed at ascertaining the effects of a single initial application of N and P on birch on the proportion of root tips colonized by ectomycorrhiza. A negative relationship between application of fertilizers

Table 3. Soil data from the experimental sites.

Site	Depth, cm	pH	C, g kg^{-1}	N, g kg^{-1}	C/N	P, mg kg^{-1}	K^+, cmol(+) kg^{-1}
Markarfljótsaurar	0-15	5.9	1.0	0.10	10.0	0.52	0.29
Kollabær	0-5	5.4	108.0	6.50	16.6	0.47	1.05
	6-15	5.7	64.8	5.42	11.9	0.25	0.71
	30-45	6.1	58.5	4.96	11.8	0.10	0.19

and ectomycorrhizal colonization has been established in several studies (e.g. Wallander 1995, Wallenda & Kottke 1998, Allen et al. 2003, Treseder 2004). In our study the larger applications of N led to a decrease in ectomycorrhizal colonization in the first year at the rich K-site, as expected (Figure 1A). However, at the poor M-site only the largest N application (N8P25) decreased the ectomycorrhizal colonization significantly, to a level where it was almost non-existent (Figure 1A). These same seedlings however showed the greatest growth response (Figure 2A). This is in accordance with earlier studies on birch (Newton & Pigott 1991b).

At the rich K-site the application of N alone had no significant effect compared to control seedlings, but at the poor M-site, a slight increase in colonization resulted (Figure 1A). This may indicate that not only tree growth but also the ectomycorrhiza might be N-limited in nutrient poor Andisols in Iceland. In a study of *Betula nana* on two arctic tundra ecosystems Clemmensen et al. (2006) found that the ectomycorrhizal biomass increased after addition of NPK fertilizer, which was explained both with increased biomass of the ectomycorrhizal plants and by increased fungal growth as a result of the treatment. In the present study P alone had no effect on the ectomycorrhizal colonization after one year at the poor M-site, while at the rich K-site there was a slightly non-significant negative tendency (Figure 1A).

The seedlings receiving only nitrogen had the lowest foliar P/N values (Table 2), which can be interpreted as a deficiency of phosphorus. These also had the highest ectomycorrhizal colonization at the poor M-site (Figure 1A), which is in accordance with earlier results that severe P deficiency strongly stimulates mycorrhizal development due to increased host carbohydrate pools (Wallander & Nylund 1992).

Several hypotheses have been presented to explain the negative effect of N and P application on ectomycorrhizal development. In a review Wallander (1995) concludes that the reduction of mycorrhizal fungi following N fertilization might be because the mycorrhizal fungi itself allocates more carbon to the process of N assimilation. Further, that it is the fungus rather than the host that modifies its carbon allocation patterns to suit the N supply situation, resulting in a reduced rate of ectomycorrhizal growth at elevated levels of N supply. This is also in accordance with Bidartondo et al. (2001) who suggested that a high carbon cost of ammonium uptake of the mycelium was one explanation for reduced sporocarp production and mycelial growth by ectomycorrhizal fungi commonly found after high levels of nitrogen addition.

The negative effect of a single large NP initial fertilization on ectomycorrhizal colonization in the present study was a temporary effect that had mostly disappeared after only three years. Tétreault et al. (1978) also found the negative effect of N application to ectomycorrhizal colonization on balsam fir seedlings to be temporary, as it increased again if the seedling N supply was decreased to the original level. Wallander and Nylund (1992) suggested that nitrogen fertilization on forest land would only have a passing effect on the ectomycorrhizal development. Furthermore, in a recent study on mature Norway spruce in a long-term experiment with annual additions of fertilizer, the number of ectomycorrhizal connections was not affected after 15 years of treatment (Fransson et al. 2000). This shows how transient the initial decrease in ectomycorrhizal colonization due to fertilizer application may perhaps be. The proportion of colonized root tips at the poor M-site was on average higher than at the rich K-site three years after planting. This finding is in accordance with recent studies showing a higher ectomycorrhizal biomass in a boreal forest ecosystem (Nilsson et al. 2006) and ectomycorrhizal colonization on i.e. *Betula pendula* Roth (Dehlin et al. 2004) on soils with a lower nutrient availability than on more fertile soils.

There were three possible sources of ectomycorrhizal fungal inoculum for the birch seedlings in the present study: a) spores that were dispersed from nearby fungal sporocarps, b) fungi attached to roots of vegetation on the site, and c) fungi that colonized the roots of the seedlings in the nursery. The greater variation in ectomycorrhizal morphotypes that was found at the rich K-site (Figure 1) might indicate a larger variation in naturally occurring plants at that site. In a study looking at plant growth and ectomycorrhiza formation on deglaciated land in Alaska, Helm et al. (1999) concluded that the ectomycorrhiza found on planted seedlings were a subset of those found on naturally occurring plants at the site. This might explain the uniformity of ectomycorrhizal morphotypes at Markarfljótsaurar (Figure 1) as there was very limited vegetation at the site. Samples were not taken of the seedlings in the nursery, but it is likely that all seedlings were inoculated with ectomycorrhiza commonly found at nurseries (Oskarsson Ú. pers. comm).

At the rich K-site there was a trend towards lower ectomycorrhizal colonization on the seedlings receiving low N with P three years after planting and fertilization (Figure 1B). A study with eucalyptus seedlings showed that high N levels combined with low P levels increased mycorrhizal colonization (Mason et al. 2000). It cannot be concluded from our results whether such NP ratios have any effect, especially because N alone does not show this effect (Figure 1B).

Survival and frost heaving

Survival was not affected by the fertilizer application. The largest dosages contained 8 g N and 10.6 g P (80 g fertilizer) per seedling. Such large dosages were expected to increase mortality, due to the high osmotic effect of the high salt concentrations in the rooting zone, which may inhibit the water uptake of seedlings (Brockley 1988). This was however not the case since there was sufficient precipitation during the weeks after planting (pers. comm. Icelandic Meteorological Office 2000). In a similar study in southern Iceland, fast-release NP fertilizer, 3.7 g N and 1.4 g P per seedling, increased mortality significantly in the first growing season, when seedlings were exposed to drought during the first weeks after planting (Oskarsson et al. 2006).

Frost heaving is regarded as one of the main factors affecting the survival of tree seedlings during the first winters after planting in Iceland (i.e. Aradóttir 1991), but fertilization at the time of planting has been found to reduce the effect (Oskarsson et al. 2006). The smaller dosages in the present study were therefore expected to have a positive effect on survival. However, only very few birch seedlings were lifted by frost heaving in the trial at the K-site and none at the M-site. Permanent snow cover reduces the frost heaving of tree seedlings (Bergsten et al. 2001), which might have been the case in the present study. The first winter after planting (1998-1999) was rather cold with permanent snow cover for a long period (pers. comm. Icelandic Meteorological Office 2000).

Growth factors

The aim of initial fertilization in afforestation in Iceland is not mainly to increase growth but rather to vitalize the tree seedlings and alleviate the stressful conditions the seedlings are exposed to. Rapid seedling growth during the first growing season increases the survival rate during the first winter (Brockley 1988).

Control seedlings and seedlings only receiving P were the smallest (Figure 2), which indicates that N is the main limiting nutrient in these soils. This is in accordance with earlier results (Óskarsson & Sigurgeirsson 2001). However, the growth responses to N fertilization were amplified when combined with P. This result indicates that P is also in short supply, and can easily become the limiting nutrient if only N is applied.

The highest N dosages (8 g N) with P had the largest effect on seedling dry weight at the poor M-site. However, at the rich K-site the smaller NP dosages, 1-4 g N, gave effects similar to those of the largest ones (Figure 2). Also, after one year the difference in dry weight between the treatments N1P15 and N8P25 was significant at the poor M-site, while at the more fertile site there was no difference between these treatments (Figure 2A). This difference in the response to the initial fertilization was even clearer three years after planting (Figure 2B). The fact that fertilization at the poor M-site gave a higher overall growth response than at the rich K-site and why the difference between fertilizer treatments N0.5 to N8 was smaller might be explained by the following. At the sparsely vegetated M-site, the birch seedlings had negligible competition from the local vegetation, while at the rich K-site the plowed furrows were surrounded by grass which could have competed with the birch for nutrients. The largest fertilizer dosages might therefore have benefited the local vegetation more than the birch seedlings, which because of small initial root systems, could not have absorbed all the applied nutrients.

Foliar nutrient concentration

The nutrient concentration of leaves is a good indicator of the nutrient status of whole plants (Marschner 1995). However short-term fluctuations in foliar nutrient concentrations, e.g. dilution effects by fast growth or accumulation of carbohydrates in leaves, might complicate the interpretation of foliar analyses (Marschner 1995). Foliar samples in this study were collected in late August, and such dilution effects should not be expected at that time as the most active growing period (June-July) had passed.

Fertilization at the time of planting increased the foliar nitrogen concentration by 360% and 290%, and the phosphorus concentration by 770% and 280%, on the poor M- and rich K-site, respectively (Table 2). This increase in foliar nitrogen and phosphorus concentrations was larger than previously seen in field studies with birch in Iceland (Oskarsson et al. 2006, Hreinn Óskarsson unpublished results). However, the dosages applied in those studies were smaller than in the present study. Control seedlings and those only receiving phosphorus were deficient in nitrogen (Table 2), while other treatments were within N sufficiency limits, according to van den Burg (1990). These results reflect the low availability of nitrogen at the poor M-site as well as at the rich K-site.

The target N ratios in Table 2 show the relative levels of nutrients (N=100) that may be considered to be suitable for optimum nutrition of birch seedlings in culture solutions (Ingestad 1971, Ingestad 1979, Ericsson & Ingestad 1988). Comparison of these to the measured foliar values revealed low ratios of P/N, K/N, S/N, Cu/N and B/N (Table 2). This indicates that these elements would be the next candidates for nutrient limitations, if N is added to the system. Similar results were also obtained by Sigurdsson (2001) in a study on black cottonwood saplings (Populus trichocarpa [Torr. and Gray]). The ratios of Ca/N, Mg/N, Mn/N, Fe/N and Zn/N were, however, rather high (Table 2). The low nitrogen concentration found in control seedlings and those only receiving P shows that N is the main limiting nutrient; however N should always be applied with P, K, S, Cu and B.

Soil properties

The experiment was performed on two contrasting sites with similar climate conditions. Soils on both sites are classified as Andisols, a volcanic soil type which covers approximately 80% of Iceland (Arnalds 2004). The soil at the poor M-site is about 75 years old and is composed of gravel and sand deposits from the River Markarfljót. It is extremely deficient in organic matter and nitrogen, while at the rich K-site the soil would be considered as rather fertile in comparison with other chemical analyses in Iceland (Johannesson 1960) (Table 3). These two sites are representative of large parts of Iceland as the fully vegetated areas typically have Andisols with large amounts of organic carbon whereas eroded areas are barren deserts with little organic carbon (Oskarsson et al. 2004).

The high N and C concentration down through the soil profile at the rich K-site is characteristic of soils that have had constant thickening due to wind-borne deposits and slow decomposition of organic material. The low temperature, deep root penetration and the presence of allophone clay may hamper decomposition of organic material in such soils (Arnalds et al. 1995). The P concentration was slightly higher at the poor M-site (Table 3), which might be explained by the lack of strong immobilizers there, such as of complexes of organic material, iron and aluminum. At the K-site more immobilizers, in addition to allophane clay minerals, which also cause P retention in Andisols, could have made the chemical extraction of P and the subsequent analyses difficult (Arnalds 1998).

CONCLUSION

Fast initial growth is important when establishing birch seedlings on cold and infertile Andisols in Iceland. Initial fertilization has been shown to have a positive effect on survival and growth. Our results show that initial fertilization can reduce ectomycorrhizal colonization if fertilizer is applied in large dosages. This reduction in ectomycorrhiza is temporary, however, and the roots of birch seedlings are colonized only a few years after the initial fertilization.

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