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A COMPARISON OF FUNGAL FLORAS OF HIGHLAND AND LOWLAND PASTURE IN ICELAND[1]

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Fifty-four soil samples were collected during the summers of 1982 nd 1983 from a reclaimed bogland (elevation 20 m = lowland) pasture at approximately 64°N, 21°W and 40 soil samples were obtained in the same time period from a highland (elevation 474 m = highland) pasture at about 64°N, 19°W. The mean number of fungal propagules per gram of soil of the former was 5.3×10^5 and of the latter 2.9×10^5 and the difference was significant at the $P < 0.001$ level. The species present in the two floras were different. *Gliocladium catenulatum*, *Paecilomyces carneus* and *Pseudeurotium zonatum* were present in more than 60% of the lowland soil samples and accounted for 21% of the total flora, but were not found in the highland pasture soil. Conversely, the most common species found in the highland soil *Penicillium* sp. 525 was not isolated from the lowland soil samples.

Cinquante-quatre (54) échantillons de sol ont été récoltés d'un pâturage réclamé d'un terrain marécageux (élévation 20 m = bas terrain) situé à 64°N, 21°W au cours des étés de 1982 et 1983. Quarante échantillons (40) de sol ont été obtenus d'un pâturage élevé (élévation 474 m = haut terrain) situé à 65°N, 19°W pendant la même période. La quantité moyenne de propagules fongiques de sol provenant du haut et du bas terrain était respectivement de 5.3×10^5 et 2.9×10^5 par gramme de sol. La différence est significative au niveau de $P < 0.001$. Les espèces présentes dans les deux flores étaient différentes. *Gliocladium catenulatum*, *Paecilomyces carneus* et *Pseudeurotium zonatum* étaient présentes dans plus de 60% des échantillons provenant du bas terrain et constituaient 21% du total des flores, mais elles n'ont pas été trouvées dans les échantillons provenant du haut terrain. Inversement, l'espèce la plus commune dans les échantillons provenant du haut terrain, *Penicillium* sp. 525, n'était pas présente dans les échantillons provenant du bas terrain.

Introduction

It has been shown that the daily weight gain of lambs on highland pastures in Iceland is about 270 g while the gain of similar lambs on reclaimed bogland in southern Iceland is about 230 g and that this difference is statistically highly significant and extends to the weight and quality of the carcasses (Gudmundsson, 1987). The reasons for this difference are unknown.

Recently evidence has accummulated which indicates that metabolites of several fungi from the soils of permanent pasture affect the metabolism of bacteria that are functionally important in digestion of feed in the rumen (Brewer *et al.*, 1979; Jen and Jones, 1983; Liss *et al.*, 1985). Thus it appeared that the ill-thrift phenomenon in Iceland might be due in part to such an interaction. We have therefore examined the fungal flora of soil which supported herbage where ill-thrift was common and compared it with the flora of soil of pasture where good performance was achieved. The results of these studies are reported.

Materials and Methods

Collection of soil samples. - The geographical location of the experimental plots is shown on the maps (Fig 1A and Fig 1B). Each plot was divided into 10 sectors as shown in Fig 2A for the lowland plot and Fig 2B for the highland. Samples were collected from 5 sectors of each experimental plot as indicated in Tables I and III, except for the

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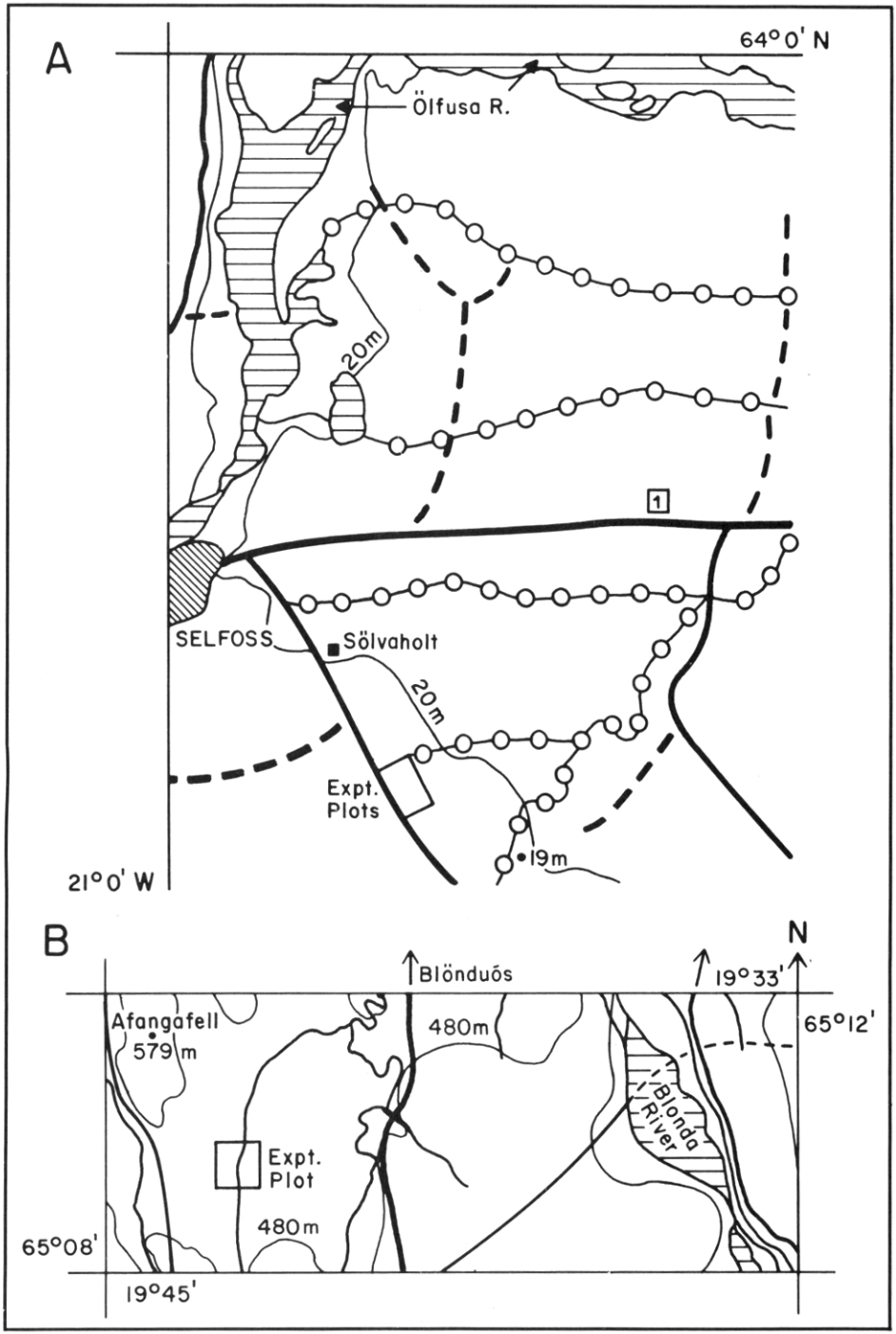

Fig 1 A. Map (1:100,000) of location of lowland plot; —— 20 m contour, —○— drainage ditches, river, [——] roads, [– – –] track; B. Map (1:100,000) of location of highland plot. The maps are based on the Iceland 1:100,000 series with much detail omitted from the drawings.

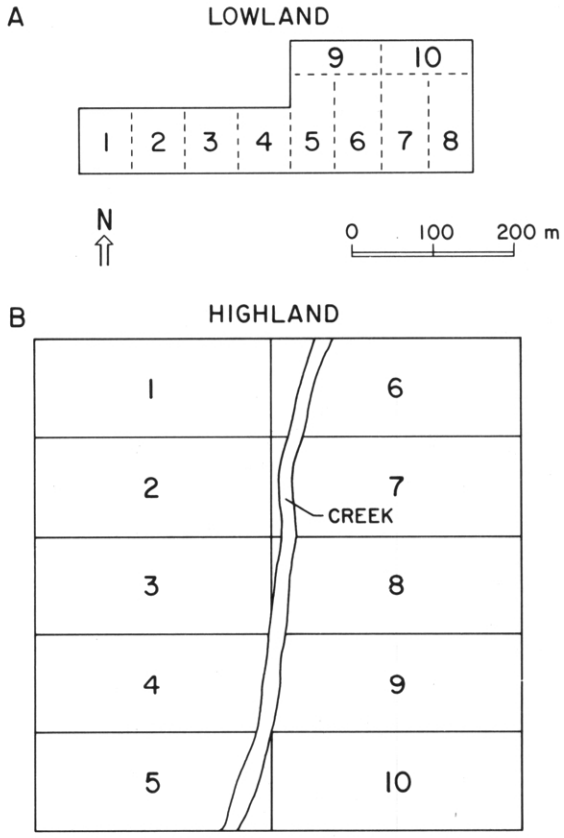

Fig 2 A. Location of sectors on lowland plot; B. Location of sectors on highland plot.

initial sampling of the lowland plot, where only 4 samples were collected. The sectors from which soil cores were obtained on particular dates were randomly selected and the method of collection was identical to that described by Brewer *et al.*, 1971. At the time of collection, the soil temperatures at the 1 and 5 cm depths were recorded. The rainfall occurring in the 3 weeks prior to the first sampling date of each year and thereafter the rainfall between sampling dates was recorded.

Collection of fungi from the herbage of experimental plots. - Fungal spores were collected from the herbage by means of a spore trap. A drawing of the trap is given in Fig 3. The trap was operated by sucking air through the filters whilst walking on the pasture. The trap was swung laterally during collection so that the rubber flaps disturbed the herbage. It was calibrated by calculating the volume of air sucked through the filters by one stoke of the pump. Each filter from the trap was washed with distilled water (50 mL), the spores dispersed by shaking and aliquots (1-5 mL) were filtered through filter discs of diameter 13 mm and porosity 1.2 μm (Millipore Corporation, Bedford, Mass.). The resulting filter discs were air-dried, mounted in 70% lactic acid on microscope slides and all the spores on each disc were counted. This number was converted to the number of spores found in each litre of air passed through the trap at standard temperature and pressure.

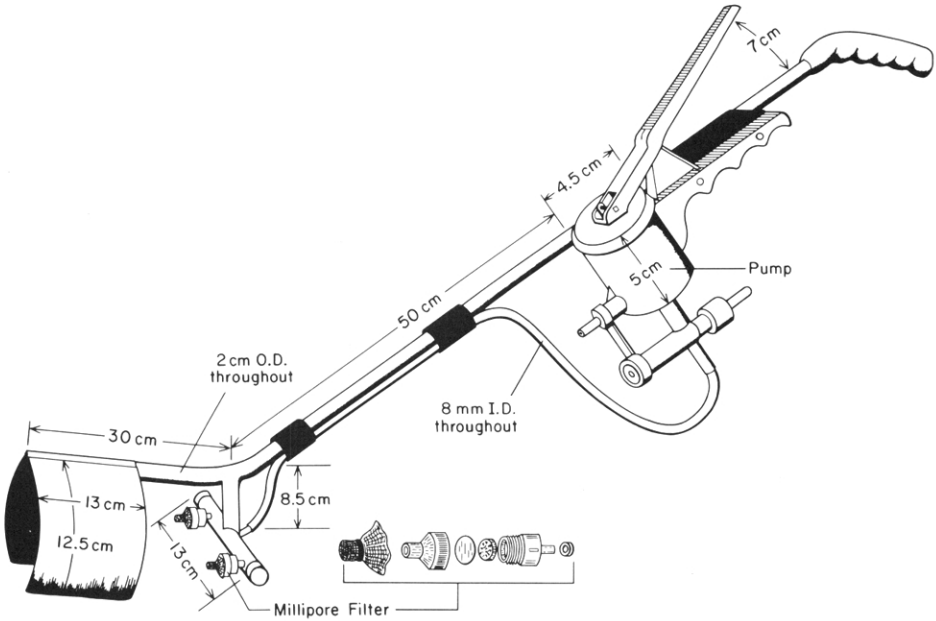

Fig 3 Diagram of spore trap.

Isolation of fungal propagules. - The time that elapsed between the collection of the samples and the dilution plating was 3 to 4 hours for the lowland and 15 to 20 hours for the highland samples.

The method of dilution plating was described by Brewer et al., 1971, with the addition to the medium of soluble starch (1 g L^{-1}) and cellulose (6 g L^{-1}, MN300, Nagel & Co). The plates were incubated for 8 to 12 days at 25°C and a subculture was made from each colony that appeared to be different from any other colonies. The number of colonies of each type that occurred on the 10 plates of the dilution from which the subculture was made (10^5 or 10^4) was recorded together with the ability to hydrolyze starch and cellulose, the ability to reduce rose bengal, and the growth-inhibiting effect (if any) on adjacent fungal colonies (Brewer and Taylor, 1980). After incubation of 1 to 2 weeks, the cultures were sent to the Atlantic Research Laboratory for identification.

Recording of field data, identity and physiological characteristics of fungal populations. - The field data in the previous paragraph and meteorological data were recorded in field books as previously described (Brewer and Taylor, 1980). This information together with the results of taxonomic studies and antibiotic screening were transferred to machine readable form as described (Brewer and Taylor, 1980). All computations were done on a Control Data Cyber 170-730 computer. Some of the programs used have been published (Brewer & Taylor, 1980). Statistical analyses of the data were performed using programs in the BMDP (Dixon, 1985) package.

Selection of random samples. - The data recorded in the field books was used to assemble a theoretical soil fungal population based on the frequency of morphologically distinct colonies on the isolation plates. Six different samples each of 150 isolates were selected at random from this theoretical population and the similarity of each random sample to the total population scored. This procedure applied to the floras of both the highland and lowland plots has been described in detail (Brewer and Taylor, 1980).

Growth inhibitory activity of the isolates in random samples. - All viable cultures in the random samples from the two plots were grown in petri dishes on the following medium: molasses 20 g, dextrin 30 g, fish meal 15 g, "Pharmamedia" (Traders Protein Division, Forth Worth, Texas) 15 g, agar 20 g and distilled water 1000 mL. The isolates were screened for antibiotic production according to the method of Brewer *et al.*, 1974. This assay employed *Micrococcus luteus* (HLX 701)* and *Candida utilis* (HLX 910), the media and temperatures of incubation of the assay organism were nutrient agar (Difco) at 35°C (*M. luteus*) and 2% malt agar at 25°C (*C. utilis*).

Results

The meteorological data in Fig 4 show that in both years of this experiment the two experimental plots experienced soil temperatures of about 10° and rainfall exceeding 20 mm month^{-1} (generally 50 mm month^{-1}) over the period June to September. These

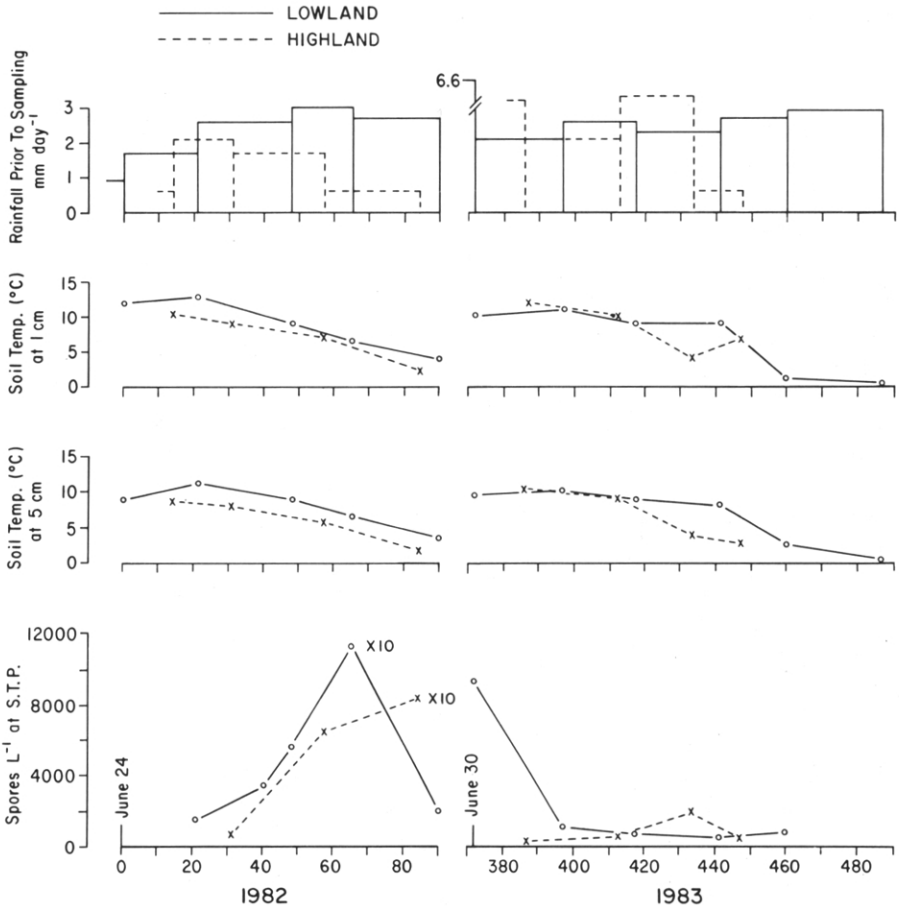

Fig 4 Soil temperatures, rainfall and spore numbers per litre of air on lowland and highland plots 1982 and 1983.

* Accession No. to culture collection at the Atlantic Research Laboratory.

Table I Dates of collection from the lowland plot indicating the sectors sampled and the calculated number of propagules per gram of soil (x 10^{-4}).

Day No.	Date	Sector Number 1	2	3	4
1	24/6/82				57
21	14/7/82	76	24 (H)		29
48	10/8/82	43			40 (H)
65	27/8/82	43		17	
90	21/9/82	31 (H)		36	
372	30/6/83		25	16 (H)	37
397	25/7/83		42		
418	15/8/83		48		162
442	8/9/83		30		
461	27/9/83		18	38 (H)	
488	24/10/83		43		115 (H)
Mean ± SD		48	33 ± 11	27	73 ± 53
No. of isolates in collection		116	201	94	161

benign conditions led inevitably to fungal growth and this is clearly demonstrated in Fig 4 where fungal efflorescences resulted in spore densities greater than 10^5 L^{-1} air on the lowland pasture and 8 x 10^4 on the highland.

Fungal populations of the soil of experimental plots

Lowland Plot. Fifty four soil samples were collected from this plot on 11 collection days. The dates of collection and the sectors of the plot from which they were acquired are indicated in Table I. The mean number of propagules collected in each gram of soil was 5.3 x 10^5 ± 3.5 x 10^5. The mean values for each sector are also given in Table I and although few samples were collected it is clear from the standard deviations that there were no differences among those sectors from which 6 or more samples were obtained. We thought that there might be differences in the fungal populations of the soil beneath the hummocks (samples in Table I marked (H)) in the experimental plots and the flora from that of the flat pasture. However, there appears

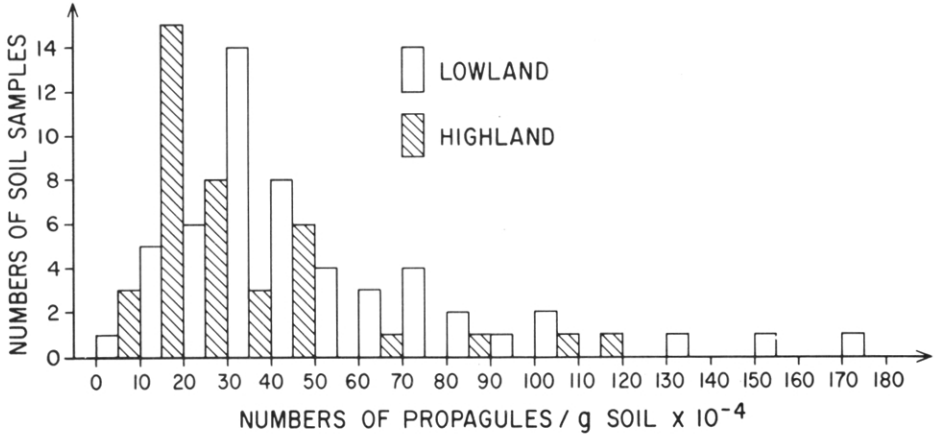

Fig 5 Histograms of numbers of fungal propagules per gram of dry soil collected in 1982 and 1983 on highland and lowland pastures.

Sector Number					
5	6	7	8	9	10
		26	37	9	
			19 (H)		102
	25	36 (H)			39
	76 (H)			15 (H)	46
		33 (H)	61 (H)		70 (H)
		57 (H)	50		
81 (H)		39	61 (H)	58	
33 (H)		75	44 (H)		
	88	29	106 (H)	39 (H)	
	32 (H)		64		38 (H)
135		90 (H)	172 (H)		
81	48	48 ± 24	68 ± 46	37	59
110	95	260	277	114	161

to be no difference in the two populations (mean number $5.5 \times 10^5 \pm 3.7 \times 10^5$, n = 24 under the hummocks and $5.1 \times 10^5 \pm 3.4 \times 10^5$, n = 30 on flat pasture). The data in Table I are also shown as a histogram in Fig 5 to illustrate the distribution of the population in groups of 10^5. When these results were subjected to a linear least mean squares test the gradient of the fitted line was 0.06 and it may be concluded that a change in population numbers during the experimental period did not occur.

The number of fungal isolates cultivated from these fifty-four soil samples was 1589 and their mean frequency was 1.8×10^5. Twenty-eight percent of these isolates have been classified with respect to species, or genus or have been assigned a taxonomic number on the basis of their characteristic morphology. A list of these fungi is given in Table II together with their frequencies, the number of soil samples in which they occurred, and the temperature range recorded for the soil sample at a depth of 1 cm when it was collected. Of these 50 species only 10 were found in 10 or more soil samples. In all cases they were isolated form soil plugs over the whole temperature range (0°-15°) and the sum of their frequencies (1.18×10^7) account for 41% of all the propagules collected (2.86×10^7). Further, four of these species *Gliocladium catenulatum, Paecilomyces carneus, Pseudeurotium zonatum and a Cylindrocarpon* sp. were found in almost 60% of the soil samples and the sum of their frequencies account for 23% of all the propagules collected.

Highland Plot. Forty soil samples were collected from this plot on 8 collection days. The dates of collections and the sectors of the plot from which they were obtained are indicated in Table III. The mean number of propagules collected in each gram of soil was $3.6 \times 10^5 \pm 5.5 \times 10^5$. Comparison of this result with that from the lowland plot using Student's "t" test showed that the variances of the two populations were the same (F value 0.79) and that the difference of the means was significant ($P < 0.1$). The number of propagules collected from sector 6 on 14/9/83 (Table III) was about 3 times greater than that of the sample (sector 8, 31/8/83) with the next highest population density on this plot and almost twice as high as that isolated from any other soil sample (lowland plot, sector 8, 14/10/83). If this high result is omitted from the analysis the mean value of propagules isolated from the highland plot becomes $2.9 \times 10^5 \pm 2.5 \times 10^5$ and the difference between the highland and the lowland plots becomes significant at the $P < .001$ level. The mean values for each sector are given in Table III and apart from the anomalous sector 6 it is clear that there were no significant differences.

Table II List of classified fungi from soil of the lowland plot, numbers of propagules per gram of soil, their ability to hydrolyse starch, reduce rose bengal and inhibit the growth of other fungi.

Species	Propagules $\bar{M}$ [a]	Number of Soil Samples	Isolates in Collection	Temp. Range °C	Activity: Antifung. $\bar{M}$ [a]	n	Starch hydrol. $\bar{M}$ [a]	n	Rose Bengal reduction $\bar{M}$ [a]	n	Known Metabolite(s)
Acremonium butyri (van Beyma) Gams	1	1	1	10-12	0	0	0	0	0	0	-
Alternaria alternata (Fr.) Keissler	22	3	4	4-15	0	0	0	0	0	0	AK-toxins
Apiospora montagnei Sacc.	12	2	2	10-12	0	0	0	0	12	2	-
Aspergillus repens De Bary	1	1	1	13-15	0	0	0	0	0	0	-
Aspergillus sp. (110)	10	1	1	13-15	0	0	10	1	0	0	
Botrytis cinerea Pers. ex Nocca & Balb	32	2	4	10-15	0	0	0	0	11	2	Botrylacton
Cephalosporiopsis sp. (794)	5	3	3	7-15	1	1	0	0	0	0	
Chaetomium cochliodes Palliser	9	2	2	4-6	0	0	0	0	0	0	Cochliodinol
Chaetomium funicolum Cooke	1	1	1	10-12	0	0	0	0	0	0	-
Chaetomium sp. (200)	10	1	1	13-15	0	0	0	0	0	0	
Cladosporium cladosporioides (Fresen.) de Vries	7	1	1	13-15	0	0	0	0	0	0	Cladosporolides
Cladosporium herbarum (Pers.) Link ex S.F. Gray	10	3	3	13-15	0	0	7	1	0	0	Mycosporins
Cladosporium sphaerospermum Penz.	20	1	1	13-15	0	0	0	0	0	0	+
Cylindrocarpon sp. (495)	21	32	46	0-15	0	0	11	2	18	15	
Epicoccum nigrum Link ex Link	1	1	1	13-15	0	0	0	0	0	0	Epicorazines
Fusarium sp. (270)	0.8	1	1	4-6	0	0	0	0	0	0	

Gliocladium catenulatum Gilm. & Abbott	94	36	67	0-15	19	1	152	2	45	5	-
Humicola fuscoatra Traaen var. *fuscoatra*	9	1	1	7-9	0	0	0	0	0	0	-
Mortierella minutissima van Teigh.	20	1	1	10-12	0	0	0	0	0	0	-
Mucor hiemalis Weimar	6	20	22	0-15	0	0	0	0	10	2	-
Oidiodendron griseum Robak	6	1	1	10-12	0	0	0	0	0	0	-
Oidiodendron tenuissimum (Peck) Hughes	12	2	2	0-9	0	0	0	0	0	0	-
Paecilomyces carneus (Duché & Mein) Brown & Smith	36	35	48	0-15	10	1	16	4	37	27	Gliotoxin
Paecilomyces sp. (180)	8	1	1	4-6	0	0	0	0	0	0	
Paecilomyces sp. (1810)	22	2	3	10-12	0	0	0	0	0	0	
Penicillium waksmanii Zaleski	1	1	1	10-12	0	0	1	1	1	1	-
Pseudeurotium zonatum van Beyma	37	33	40	0-15	2	1	0	0	20	2	Cytochalasin G
Thielavia sp. (1512)	40	27	34	0-15	41	2	77	1	38	6	
Tolypocladium cylindrosporum Gams	23	2	2	4-6	0	0	0	0	0	0	-
Trichocladium opacum (Corda) Hughes	26	25	34	0-15	0	0	18	6	10	1	-
Trichocladium sp. (4181)	22	5	6	0-12	0	0	29	1	38	1	
Trichoderma hamatum (Bon.) Bain.	15	6	8	0-15	38	1	0	0	10	2	Dermadin
Trichoderma harzianum Rifai	2	2	3	13-15	0	0	0	0	0	0	Isocyanides
Trichoderma viride Pers. ex Gray	9	10	12	0-15	0	0	0	0	9	6	Viridin
Trichoderma sp. (170)	19	17	20	0-15	0	0	0	0	21	13	
Truncatella angustata (Pers. ex Lk.) Hughes	5	6	6	4-12	0	0	1	1	0	0	-

Table II List of classified fungi from soil of the lowland plot, numbers of propagules per gram of soil, their ability to hydrolyse starch, reduce rose bengal and inhibit the growth of other fungi (continued).

Species	Propagules $\overline{M}$ [a]	Number of Soil Samples	Isolates in Collection	Temp. Range °C	Activity: Antifung. $\overline{M}$ [a]	Antifung. n	Starch hydrol. $\overline{M}$ [a]	Starch hydrol. n	Rose Bengal reduction $\overline{M}$ [a]	Rose Bengal reduction n	Known Metabolite(s)
Ulocladium atrum Preuss	1	1	1	10-12	0	0	0	0	0	0	-
Ulocladium consortiale (Thüm) Simmons	37	6	8	4-15	0	0	64	2	70	2	-
Unknown (781)	36	15	16	0-15	49	2	59	1	34	11	
Unknown (782)	12	1	1	0-3	0	0	0	0	12	1	
Unknown (783)	157	4	4	4-15	0	0	580	1	0	0	
Unknown (784)	2	1	1	10-12	0	0	0	0	0	0	
Unknown (785)	6	2	2	10-12	0	0	0	0	11	1	
Unknown (787)	2	1	1	13-15	0	0	2	1	0	0	
Unknown (788)	22	1	1	13-15	0	0	22	1	0	0	
Unknown (789)	10	4	4	7-12	0	0	0	0	0	0	
Unknown (790)	157	7	7	0-12	0	0	0	0	181	6	
Unknown (791)	114	7	8	0-12	0	0	0	0	135	4	
Verticillium cephalosporum Gams	23	3	3	0-12	0	0	0	0	0	0	-
Verticillium lecanii (Zimm.) Viégas	36	2	3	0-15	0	0	0	0	0	0	Bassianolide

[a] $\overline{M}$ = mean number of propagules per gram of soil x 10^{-3}. n = number of soil samples in which the species were found. A dash in column 13 indicates that this species has no known toxic metabolites. "+" indicates that this species has been reported to produce toxic metabolites which were not characterized.

Table III Dates of collection from highland plot indicating the sectors sampled and the calculated number of propagules per gram of soil (x 10^{-4}).

Day No.	Date	Sector Number									
		1	2	3	4	5	6	7	8	9	10
14	7/7/82	1		19	17 (H)			23		16	
31	24/7/82			23		11 (H)		12 (H)	19	18	
57	19/8/82		30 (H)	21		20		15 (H)			107 (H)
84	15/9/82	46		31	6 (H)		28 (H)	24 (H)			
387	15/7/83	11 (H)		40	21	11				84 (H)	
413	10/8/83		18 (H)	26	1			60 (H)	41		
434	31/8/83		15		49				116	40 (H)	43 (H)
448	14/9/83		10			14 (H)	342 (H)	16 (H)			32 (H)
Mean ± SD		19	18	27 ± 8	19	14	185	25 ± 18	59	40	61
No. of isolates in collection	84	127	177	110	102	54	151	73	110	84	

Table IV List of classified fungi from the soil of the highland plot, numbers of propagules per gram of soil, their ability to hydrolyse starch, reduce rose bengal and inhibit the growth of other fungi.

Species	Propagules M	Number of Soil Samples	Isolates in Collection	Temp. Range °C	Activity: Antifung. M	Antifung. n	Starch hydrol. M	Starch hydrol. n	Rose Bengal reduction M	Rose Bengal reduction n	Known Metabolite(s)
Alternaria alternata	7	5	5	4-12	0	0	9	2	0	0	Tenuazonic acid
Botrytis cinerea	5	3	3	7-12	0	0	0	0	6	1	Botrydiol
Chaetomium cochliodes	85	1	1	7-9	0	0	0	0	0	0	Chetomin
Cladosporium cladosporioides	3	1	1	10-12	0	0	0	0	0	0	Asperentin
Cladosporium herbarum	7	3	4	10-15	0	0	7	1	0	0	Mycosporins
Cladosporium sphaerospermum	7	2	3	7-15	0	0	1	1	0	0	+
Cylindrocarpon sp. (495)	4	1	2	16-18	0	0	0	0	0	0	
Fusarium sp. (270)	0.8	1	1	16-18	0	0	0	0	0	0	
Humicola fuscoatra var. *fuscoatra*	1	1	1	7-9	0	0	0	0	0	0	-
Humicola grisea Traaen	3	1	1	7-9	0	0	0	0	0	0	-
Mortierella ramanniana (Möller) Linnem.	13	1	1	10-12	0	0	0	0	0	0	-
Mortierella vinacea Dixon-Stewart	42	7	9	4-12	0	0	0	0	63	4	-
Mucor hiemalis	0.5	1	2	10-12	0	0	0	0	0	0	-
Oidiodendron truncatum Barron	7	1	1	10-12	0	0	0	0	0	0	PR 1350
Paecilomyces sp. (1809)	13	1	1	10-12	0	0	13	1	0	0	
Penicillium sp. (525)	46	8	11	0-15	0	0	57	5	100	1	
Penicillium sp. (526)	14	3	4	13-15	0	0	14	3	0	0	
Penicillium sp. (527)	18	1	1	7-9	0	0	0	0	0	0	
Penicillium notatum Westling	9	1	1	7-9	0	0	0	0	0	0	Penicillin
Tolypocladium cylindrosporum	10	2	4	4-15	0	0	0	0	0	0	-

Tolypocladium niveum (Rostrup) Bisset		3	5	5	4-12	0	0	0	0	0	0	-
Trichocladium opacum		10	19	23	4-18	0	0	13	2	0	0	-
Trichoderma polysporum (Link ex Pers.) Rifai		0.2	1	1	10-12	0	0	0	0	0	0	Cyclosporins
Truncatella angustata		5	2	2	7-12	0	0	0	0	0	0	-
Ulocladium consortiale		23	5	6	0-12	0	0	0	0	6	1	-
Unknown	*(785)*	35	1	1	10-12	0	0	0	0	35	1	
Unknown	*(786)*	7	1	1	10-12	0	0	0	0	0	0	
Unknown	*(793)*	6	1	1	10-12	0	0	0	0	0	0	
Verticillium lecanii		3	3	3	7-12	0	0	0	0	0	0	Bassianolide
Verticillium sp.	(9351)	2	1	1	7-9	0	0	0	0	0	0	
Zygorrhynchus moelleri Vuill.		6	4	4	4-15	0	0	0	0	0	0	-

Abbreviations are explained in Table II.

Table V Biological activities of numbers of unclassified isolates from soils of highland and lowland pastures

Biological Activity	Lowland		Upland	
	Total Collection	Random Sample	Total Collection	Random Sample
Starch Hydrolysis	58	6	96	11
Rose Bengal Reduction	205	31	162	26
Growth Inhibition of other soil fungi	16	4	24	8
Growth inhibition of *M. luteus*		22		7
Growth inhibition of *C. utilis*		21		10

Table VI Days of collections appearing in the random sample from the lowland pasture indicating the sectors sampled and the calculated number of fungal propagules per gram of soil ($\times 10^{-4}$).

	Sector Number									
Day No.	1	2	3	4	5	6	7	8	9	10
1				15			8	1		
21	31	4		3						37
48	12			6			4			9
65	10		4							1
90	8		13				3	19		12
372		4	2				22	1		
397		12			6		12	11	26	
418		3		6	7		21	1		
442		2				10	13	53	3	
461			2			3		28		10
488		27		78	5		42	109		
Mean ± SD	15	13 ± 10	5	22	6	6	16 ± 13	27 ± 37	14	14
No. of isolates selected	11	13	8	18	5	7	27	25	8	17
% of isolates in collection	9	6	9	11	5	7	10	9	7	11

Some of the data in Table III are shown in Fig 5 where the population is sorted into groups of 10^5 illustrating the differences between the lowland and highland populations. The results from the highland plot were subjected to a linear least mean squares regression analysis and the gradient of the line obtained, 0.07, indicates the numerical stability of the population during the experimental period.

The number of fungal isolates cultivated from these 40 soil samples was 1072 and their mean frequency was 1.4×10^5. One hundred and eleven (10%) of these isolates have been classified, as defined for the lowland plot. A list of these fungi is given in Table IV, together with their frequencies, the number of soil samples in which they were found and the temperature range recorded for the soil samples at a depth of 1 cm when it was collected. Only 6 of these 31 species were found in 5 or more of the soil samples and these 6 accounted for 9% of the total propagules collected (1.18×10^6). None of the isolates inhibited the growth of other fungi on the isolation plates and only one organism (*Penicillium* sp. 525) was active in more than one test; 11 out of 31 were demonstrated to have biological activity (Table IV). This contrasts to the biological activity of the isolates from the lowland plot, where 27 out of 50 showed some activity and 20% had activity in two or more tests (Table II). The physiological properties of the unclassified isolates are summarised in Table V. The greater ability of the highland population to hydrolyse starch (115 from highland pasture vs 89 from the lowland) was mostly a property of the unclassified fungi (highland 96, lowland 58) and all the organisms that inhibited the growth of other fungi on the isolation plates from the highland soil samples remain unclassified.

Selection of a random sample of 150 isolates from the fungi of the soil of highland and lowland pastures. - A theoretical flora of 21044 organisms was assembled from isolates from the lowland plot and 6 random selections of 150 isolates were made from this flora. The selections were scored as described (Brewer and Taylor, 1980) and the one having the highest score had the following characteristics (characters of the whole population given in parentheses): number of collection dates, 11 (11); mean frequency (isolates g^{-1}) 1.9×10^5 (1.8×10^5); gradient of linear least mean squares plot 0.016 (0.06); number of soil samples 46 (54): number of different isolates 139: geographical distribution - standard deviation of percent isolates selected for each sector 2.06; classified number of organisms 18 (50). Similarly the collection from the highland plot was expanded to 10235, the selections scored in the same way, the one

Table VII Days of collections appearing in the random sample from the highland pasture indicating the sectors sampled and the calculated number of fungal propagules per gram of soil ($\times 10^{-4}$).

	Sector Number									
Day No.	1	2	3	4	5	6	7	8	9	10
14	1		4	1			11		1	
31			7		2		3	6	10	
57		12	4		8		7			35
84	21		10			8	2			
387	1		7	8	1				17	
413		3	3				28	17		
434		1		37				69		18
448					5	93	3			3
Mean ± SD	7	5	6	15	4	8	9±10	31	9	19
No. of isolates selected	8	8	20	12	13	9	18	15	17	12
% of isolates in collection	10	6	11	11	13	17	12	21	13	14

Table VIII List of classified fungi of the random sample from the lowland collection and their biological activity.

Species	Propagules $\bar{M}$	Number of Soil Samples	Temp. Range °C	Enzyme: Starch Hydrol. $\bar{M}$	n	Enzyme: Rose Bengal reduction $\bar{M}$	n	Activity of Cultures: Growth Inhibition *M. luteus* $\bar{M}$	n	Activity of Cultures: Growth Inhibition *C. utilis* $\bar{M}$	n
Alternaria alternata	21	1	13-15	0	0	0	0	21	1	0	0
Cephalosporiopsis sp. (794)	13	1	7-9	0	0	0	0	0	0	0	0
Cylindrocarpon sp. (495)	16	3	10-15	21	1	19	2	15	2	10	1
Gliocladium catenulatum	68	8	0-15	100	1	49	2	68	6	0	0
Mucor hiemalis	13	2	7-15	0	0	14	1	0	0	0	0
Oidiodendron tenuissimum	230	1	7-9	0	0	0	0	0	0	0	0
Paecilomyces carneus	46	9	0-15	39	1	63	6	0	0	0	0
Paecilomyces sp. (1810)	22	1	10-12	0	0	0	0	22	1	0	0
Pseudeurotium zonatum	70	8	0-15	0	0	38	1	104	2	38	1
Thielavia sp. (1512)	14	3	0-15	0	0	0	0	0	0	0	0
Trichocladium opacum	83	4	0-15	0	0	0	0	38	2	36	1
Trichocladium sp. (4181)	10	1	4-6	0	0	0	0	10	1	0	0
Ulocladium consortiale	11	1	7-9	0	0	0	0	0	0	0	0
Unknown (781)	140	1	0-3	0	0	140	1	0	0	0	0
Unknown (783)	35	1	13-15	0	0	0	0	0	0	0	0
Unknown (790)	420	1	0-3	0	0	420	1	0	0	0	0
Unknown (791)	91	5	0-12	0	0	143	3	0	0	0	0
Verticillium cephalosporum	48	1	0-3	0	0	0	0	0	0	0	0

Abbreviations are explained in Table II.

Table IX List of classified fungi of the random sample from the highland collection and their biological activity.

Species		Propagules M	Number of Soil Samples	Temp. Range °C	Biological Activity of Cultures: Enzyme: Starch Hydrol. M	n	Rose Bengal reduction M	n	Growth Inhibition: *M. luteus* M	n	*C. utilis* M	n
Chaetomium cochliodes		85	1	7-9	0	0	0	0	0	0	0	0
Mortierella vinacea		57	4	4-12	0	0	66	3	0	0	0	0
Paecilomyces sp. (1809)		13	1	10-12	13	1	0	0	13	1	0	0
Penicillium sp.	(525)	55	4	0-15	55	4	100	1	100	1	0	0
Trichocladium opacum		20	5	7-12	18	1	0	0	18	1	7	1
Truncatella angustata		9	1	7-9	0	0	0	0	9	1	0	0
Ulocladium consortiale		33	3	0-9	0	0	0	0	43	1	0	0
Unknown	(785)	35	1	10-12	0	0	35	1	0	0	0	0
Verticillium lecanii		9	1	7-9	0	0	0	0	0	0	9	1

Abbreviations are explained in Table II.

chosen having the characters: number of collection dates 8 (8); mean frequency (isolates g^{-1}) 1.4×10^5 (1.4×10^5); gradient of fitted line numbers vs time 0.04 (0.07); number of soil samples, 34 (40); number of different isolates 132; geographical distribution - standard deviation, 4.06; classified number of organisms, 9 (31). The distribution of soil samples selected is given in Table VI for the lowland plot and in Table VII for the highland plot. Comparisons of the biological activities of unclassified isolates in the random samples with the total collections are given in Table V.

Biological activities of random samples of fungal isolates from highland and lowland pastures. - Of the 139 different isolates in the random sample from the lowland pasture 28 proved to be non-viable on subcultivation on the antibiotic screening medium. The remaining 111 isolates were tested for their ability to produce metabolites that inhibited growth of *M. luteus* and *C. utilis*. The results of these experiments are summarised in Table VIII for the classified fungi and in Table V for the unclassified. Thirty-seven isolates (33%) of the total random sample produced metabolites that inhibited the growth of *M. luteus* and 10 isolates (9%) the growth of *C. utilis*.

Of the 132 different isolates in the random sample selected from the highland plot, 24 proved to be non-viable on subcultivation and the remaining 108 were assayed against *M. luteus* and *C. utilis*. The results of these experiments are summarised in Table IX for the classified organisms in the random sample and in Table V for the unclassified fungi. Thirty isolates (28%) of the random sample produced metabolites that inhibited the growth of *M. luteus* and 10 isolates (9%) the growth of *C. utilis*.

Discussion

In earlier work at Nappan, Nova Scotia small differences were established in numerical, taxonomic and some physiological characters of fungal populations in the soils of experimental plots that were only a few hundred meters apart. Though these plots, which had been managed similarly for at least 50 years, differed only in that one had been reclaimed from tidal marshland, the possibility exists that the smallness of the differences reported were due to some extent to the choice of methods.

By contrast, the experimental plots used in the work reported in this paper were widely separated and consequently were different with respect to climate, soil type and vegetation cover. Hence, if methods chosen to investigate the soil floras were valid it would be expected that larger differences in the floras of the two plots in Iceland would emerge than were found at Nappan.

The number of fungi on the lowland plot was almost twice that on the highland as compared with a difference of about 30% at Nappan. Taxonomically, at Nappan 83% of the fungi that were classified were found on both plots but in Iceland only 34% of the classified fungi were common to highland and lowland areas. At Nappan (Brewer et al., 1972) there was little difference in antibiotic activity when the isolates producing antibiotics were expressed as a percentage of those tested whereas in Iceland 42% of the organisms from the lowland and 37% of those from the upland produced growth inhibiting metabolites. These data, expanded in the Tables, support the expectation that greater differences would emerge between plots in Iceland.

Some of the species of fungi named in Tables II and IV are known to produce toxic metabolities when cultivated in the laboratory. The name of such a known metabolite of a species is given in the Tables. Of course, some species are known to produce several metabolites and this is indicated either by giving a generic name or by giving the names of different metabolities where the species appears in both Tables. Full details are readily available by consultation of the public "Mycotox" file (Brewer *et al.*, 1978). Of the four fungi which are most prevalent on the lowland plot only two —*Paecilomyces carneus* and *Pseudeurotium zonatum* are known to produce antibiotics. The latter has been reported (Probst and Tamm, 1981) to produce cytochalasin G

the activity of none of these metabolites against rumen bacteria has been reported to our knowledge.

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