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Effects of fertilization and pH on communities of Collembola in pine forest soil

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The effects of urea (nitrogen only) and ash + phosphorus (no nitrogen) on populations of Collembola were studied under laboratory and field conditions. In addition to the untreated controls, ash-treatment was controlled with $Ca(OH)_2$, and urea-treatment with NH_4NO_3 in order to separate the impact of pH from that of nutrients. Several species displayed significant reactions, either positive or negative, to the treatments. The results are compared with recent studies on the effects of experimental acidification and liming. Soil acidity would appear to be an essential factor in explaining the changes observed.

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1. Introduction

Mineral fertilization is a practice commonly employed in the Nordic countries to improve forest productivity. Recent forestry plans in Finland ("Metsä 2000") include, by the end of century, a severalfold increase in the area treated annually with fertilizers. Little is known about how fertilizers affect the forest ecosystem as a whole. The present study is part of an unofficial project called the "Effects of fertilization on the forest ecosystem", being carried out jointly by several research teams. The soil zoological results of the study were preliminarily reported in Finnish (Huhta et al. 1984), and Enchytraeids have been dealt with in more detail by Huhta (1984).

Microartropods (Collembola and Acari) are numerically among the most important soil animal groups in coniferous forests. The effects of forest fertilization on Collembola have been investigated in several studies (review by Marshall 1977, Mayer-Krapoll 1963, Huhta et al. 1967, 1969, Weetman et al. 1972, Marshall 1974). However, these studies only dealt with total numbers, and in some older works the experimental and sampling design is insufficiently documented. Axelsson et al. (1973), Lohm et al. (1977) and Behan et al. (1978) have studied the effects of nitrogen fertilizers on different species.

Recently it has been shown that the acidity of soil exerts a strong influence on many collembolan species (Bååth et al. 1980, Hågvar & Abrahamsen 1980, 1984, Hågvar & Kjøndal 1981, Hågvar 1984a, b). Urea, when spread on naturally acid forest soil, causes a rapid rise in the pH value, which then gradually decreases to the original level over one or two years. The same is even more true of ashes that have a strong and long-lasting neutralizing effect. In such cases, if changes in the soil animal populations are observed after fertilization, it is not possible to conclude without further experiments whether these are induced by the change in pH or by the nutrient added.

2. Material and methods

The study sites and experimental design were described in detail by Huhta (1984). Thus, only the most essential facts are repeated here. Note that the data on Collembola do not cover all the treatments and sampling periods included in the whole study.

Field experiment 1 was established in a *Calluna*-type pine stand in Tammela, southern Finland. The study plots were 10-m squares; one of them was fertilized with ash (symbol A) (7000 kg/ha) and one with ash with apatite (A+P) (≈ 44 kg P/ha) added. Two untreated plots served as controls (C). Sampling was performed monthly during the second growing period (in 1980) after fertilization. Each sample consisted of ten 25 cm^2 units from each treated plot and 5+5 from the controls. The samples were separated into two layers, 0—3 and 3—6 cm.

Table 1. Monthly mean numbers/m^2 (sums from both layers, 0 to 6 cm) of total Collembola and the most abundant species in the field experiment 1 (Tammela). Significant differences (column *P*) from the control are indicated with *, those between A and A+P with ●, and significant season/treatment interactions with * in parentheses (*=$P<0.05$, **=$P<0.01$). Note that seasonal variation has been removed from total variation by the ANOVA.

		May	June	July	Aug.	Sept.	Mean	*P*
Willemia anophthalma Börner	Control	8240	2640	5720	8200	17600	8480	
	Ash	3480	3200	4080	6560	7080	4880	
	Ash+P	5240	1240	5480	6240	11560	5952	
Micranurida pygmaea Börner	Control	4640	2160	4280	5840	4720	4328	
	Ash	2520	6080	5760	5840	9760	5992	
	Ash+P	3840	4600	5120	14800	6520	6976	
Onychiurus absoloni (Börner)	Control	6560	1400	3720	4640	1800	3624	
	Ash	13080	4720	9200	13960	12920	10776	**
	Ash+P	8480	3640	8360	18880	13440	10560	**
Mesaphorura krausbaueri Börner	Control	27960	10160	10080	41680	6400	19256	
	Ash	14800	9600	10560	35440	23000	18680	
	Ash+P	28400	15840	16680	33560	25600	24016	
Pseudanurophorus binoculatus Kseneman	Control	120	120	640	680	480	408	
	Ash	5680	1240	8520	12120	5520	6616	**
	Ash+P	720	80	400	2640	640	896	●●
Anurophorus septentrionalis Palissa	Control	11960	7440	12000	19800	13600	12960	
	Ash	16600	6400	15920	22000	15480	15280	●●
	Ash+P	3880	2440	11560	9600	9640	7424	**
Folsomia quadrioculata (Tullberg)	Control	600	800	1000	1000	80	696	
	Ash	15480	14320	11560	8800	9680	11968	**
	Ash+P	5840	5400	11120	7560	6280	7240	**
Isotomiella minor (Schäffer)	Control	31440	29200	30680	25440	16800	26712	
	Ash	20920	8960	19160	19320	27000	19072	*
	Ash+P	9800	9680	19120	16040	22560	15440	**(*)
Isotoma notabilis Schäffer	Control	320	440	40	160	200	232	
	Ash	1120	520	840	1320	2840	1328	**
	Ash+P	2440	1660	760	4080	7880	3344	**
Lepidocyrtus lignorum (Fabricius)	Control	520	1200	240	160	160	456	
	Ash	3480	1240	680	360	280	1208	
	Ash+P	1760	1640	280	360	600	928	
Megalothorax minimus Willem	Control	0	0	1680	1240	2160	1016	
	Ash	0	0	1200	1600	2120	1184	
	Ash+P	0	80	1120	680	3560	1088	
Total Collembola	Control	94880	61080	72400	111440	66000	81160	
	Ash	99840	57160	93160	132320	123000	101096	
	Ash+P	72040	47560	81880	118560	111720	86352	
Total biomass, mg d.w.	Control	192.7	89.0	77.4	134.7	109.8	120.7	
	Ash	186.9	90.8	152.5	181.2	224.2	167.1	
	Ash+P	78.0	54.4	103.0	139.6	186.1	112.2	

Field experiment 2 was performed in a young pine stand at Ruotsinkylä near Helsinki. Ten 4×4 m test plots were divided into four quadrats, each receiving randomly one of the four treatments: 1) (A+P) 2×3350 kg/ha) ashes with 2×500 kg superphosphate (total 88 kg P/ha) added; 2) (L) 2×2000 kg/ha slaked lime ($Ca(OH)_2$); 3) urea (U), 2×230 kg N/ha; and 4) (C) untreated control. The fertilizers were given in two separate applications (13 May and 1 June 1981). Samples (one unit from each 10×4 subplots) were taken in Sept. 1981, May and July 1982, and May 1983.

Two *laboratory experiments* were especially planned to separate the effects of pH from those caused by the addition of nutrients. Six intact blocks of soil, including vegetation and the topmost 1 cm of mineral soil, were placed in plastic boxes measuring 40×60 cm, depth 11 cm. Organic soil was taken from the same site, sieved with a 10 mm mesh, mixed well and divided for the different treatments. The chemicals to be tested were weighed and mixed thoroughly with this soil. Equal portions of each treatment were then placed in small mesh baskets (mesh 1.5 mm, depth 8 cm, diameter 4 cm), which were inserted into holes of corresponding size bored in the soil blocks in the boxes. These were then covered with perforated plastic,

Table 2. Mean numbers of Collembola /m^2 (sums of the layers 0–3 and 3–6 cm) in field experiment 2 (Ruotsinkylä). Significant differences are indicated (* and ** between the treatment and the control in samples from 1982 and 1983; o and oo the same in Sept. 1981 only; ● between urea and ash, (*) treatment/season interaction; see Table 1 for other explanations).

		Sept. -81	May -82	July -82	May -83	Mean	*P*
Willemia anophthalma Börner	Control	1960	954	2332	2438	1921	
	Ash	1880	318	6254	1166	2405	
	Lime	2760	212	424	1166	1141	*
	Urea	1440	2356	318	212	1082	
Micranurida pygmaea Börner	Control	6680	8162	11872	5724	6272	
	Ash	16520	9964	5830	4346	9165	
	Lime	5640	13886	4464	1802	6448	
	Urea	1560	2944	1802	848	1788	**
Onychiurus absoloni (Börner)	Control	800	0	848	212	465	
	Ash	120	212	742	742	454	
	Lime	640	1272	1602	636	1038	
	Urea	520	329	530	106	501	
Mesaphorura krausbaueri Börner	Control	28600	17172	17808	9646	18307	
	Ash	31000	13144	12084	22896	19781	
	Lime	22960	13250	14039	19292	17385	
	Urea	11960	13662	19928	11766	14329	
Folsomia fimetarioides (Axelson)	Control	23360	31588	15264	11766	20495	
	Ash	54360	34768	29468	7632	31557	oo
	Lime	38400	23320	23238	17066	25506	
	Urea	34360	9363	20246	14734	19676	
Isotomiella minor (Schäffer)	Control	10280	14204	16854	7208	12137	
	Ash	10800	19186	13780	13992	14440	
	Lime	8649	28090	26052	14946	19434	*
	Urea	5840	10906	12508	13674	10732	
Isotoma notabilis Schäffer	Control	2160	6148	2968	2120	3349	
	Ash	3960	11660	8480	4134	7058	*
	Lime	4360	10600	7161	8268	7597	**
	Urea	0	3792	4346	3392	2883	oo
Megalothorax minimus Willem	Control	6680	1166	4770	0	3154	
	Ash	2560	1378	848	0	1197	(*)
	Lime	4400	2544	2356	0	2325	
	Urea	1320	2344	2862	0	1632	oo
Total Collembola	Control	84960	90336	74306	41234	72709	
	Ash	123240	92008	79818	57240	88077	
	Lime	95080	94870	81761	65402	84278	
	Urea	64600	45215	64978	48442	55809	●
Total biomass, mg d.w.	Control	96.9	117.1	76.9	75.4	91.6	
	Ash	143.2	119.8	109.5	58.3	107.7	
	Lime	132.2	103.6	111.2	95.5	110.6	
	Urea	112.6	60.5	102.7	79.0	88.7	

kept moist by watering and incubated at a fluctuating temperature (day +20°C, night +15°C). Both experiments were interrupted by a simulated winter: the temperature was gradually lowered to close to freezing point for approx. 2 months. The treatments were:

Experiment 1: (A+P) Birch ashes, 9.7 g, with superphosphate added, 1.4 g/kg (f.w.) of soil, equivalent to 1750 kg ashes and 22 kg P/kg; (L) slaked lime, $Ca(OH)_2$, 9.7 g/kg; (C) control without fertilizers.

Experiment 2: Urea (U), 2.6 g/kg (f.w.) of soil; ammonium nitrate (AN), 3.4 g/kg, both equivalent to 150 kg N/ha; control (C) without addition of N.

At selected intervals, six randomly chosen replicates (one from each box) of each treatment were removed for estimating the numbers of microarthropods.

Animals were extracted from the samples with a "high gradient" canister extractor, applying the "medium" heating regime recommended by Leinaas (1978) for raw humus soil.

Mesaphorura krausbaueri Börner in the field data actually includes three species: *M. yosii* (Rusek), *M. macrochaeta* Rusek and *Karlstejnia norvegica* Fjellberg, of which *M. yosii* was most abundant. In some selected samples from Tammela (control plots) *K. norvegica* contributed less than 5% to *M. krausbaueri* s.l., and only one specimen of *M. macrochaeta* was found. At Ruotsin-

Table 3. Numbers of the most abundant species of Collembola (totals of six replicates) in the laboratory experiments. Significant differences are indicated (* and ** between treatment and control, ● and ●● between urea and NH_4NO_3).

		M. pygmaea	*O. absoloni*	*M. yosii*	*F. fimet.*	*I. minor*	*I. notab.*
Experiment 1							
5 October	Control	246	9	414	201	281	88
	Ash	31**	8	141*	152	190	302*
	Lime	26**	24	145*	148	284	345*
1 December	Control	823	4	259	765	633	131
	Ash	194	76	58	202*	676	276*
	Lime	126	296	64	192*	708	254
9 March	Control	437	17	274	797	514	112
	Ash	84	64*	232	890	618	206
	Lime	82	134**	164	1028	378	144
17 May	Control	72	34	90	793	26	8
	Ash	10	90	46	84**	40	34*
	Lime	2	142	88	66**	22	26
Experiment 2							
25 May	Control	0	0	15	63	17	2
	Urea	1*	0	2*	8**	0*	0
	NH_4NO_3	0	0	3*	36**	2*	0
14 June	Control	6	2	475	49	307	40
	Urea	0**	1	352	14**	214	61
	NH_4NO_3	1**●●	0	130**●●	1**	5*	17
5 July	Control	6	2	802	200	264	55
	Urea	1*	2	468	1**	258	53
	NH_4NO_3	3	1	247**●●	0**	5**●●	28
27 July	Control	24	2	516	138	152	50
	Urea	2*	0	189	13**	195	82
	NH_4NO_3	4	6	367	0**	4**●●	34●
7 September	Control	56	12	715	230	178	54
	Urea	34	18	304*	166	341	34
	NH_4NO_3	10	4	322	58	54	30
7 December	Control	11	12	136	235	114	16
	Urea	6	8	131	169	245	25
	NH_4NO_3	6	47	204	153	60	30

kylä (control) *K. norvegica* was not found and *M. macrochaeta* contributed approx. 5 %. *Folsomia quadrioculata* (Tullberg) is here treated in *sensu lato*.

The biomasses were estimated using the regression formulae given by Tanaka (1970) and Petersen (1975).

The differences between the treatments and their respective controls have been tested with the aid of two-way analysis of variance after logarithmic transformation (field experiments), using treatment and season (or no. of sample) as factors. As preliminary examination provided no reason for treating the horizons 0–3 and 3–6 cm separately, only the sums from both horizons were tested. In field Exp. 2, the first sample (Sept. 1981) was tested separately from the others, because it seemed that the effects of the treatments had not yet appeared. Student's *t*-test from original figures for each sample separately was used for the laboratory experiments.

3. Results

3.1. Field experiments

The ash treatments did not affect the total numbers of Collembola, but populations of several individual species showed significant changes, especially in Exp. 1 (Table 1). *O. absoloni, F. quadrioculata* and *I. notabilis* were found in higher densities in both ash-treated plots, while *I. minor* displayed an opposite reaction. *P. binoculatus* and *A. septentrionalis* showed significant differences in one of the two treatments. In Exp. 2, significant differences after ash-treatment were recorded in two cases only; *F. fimetarioides* and *I. notabilis* showed increasing trends (Table 2). Comparison between these experiments is difficult owing to the different species composition in the two experimental fields: most species that reacted significantly in one experiment were rare in the other. On the other hand there were several species common to both experiments which did not react significantly in either: *W. anophthalma, M.*

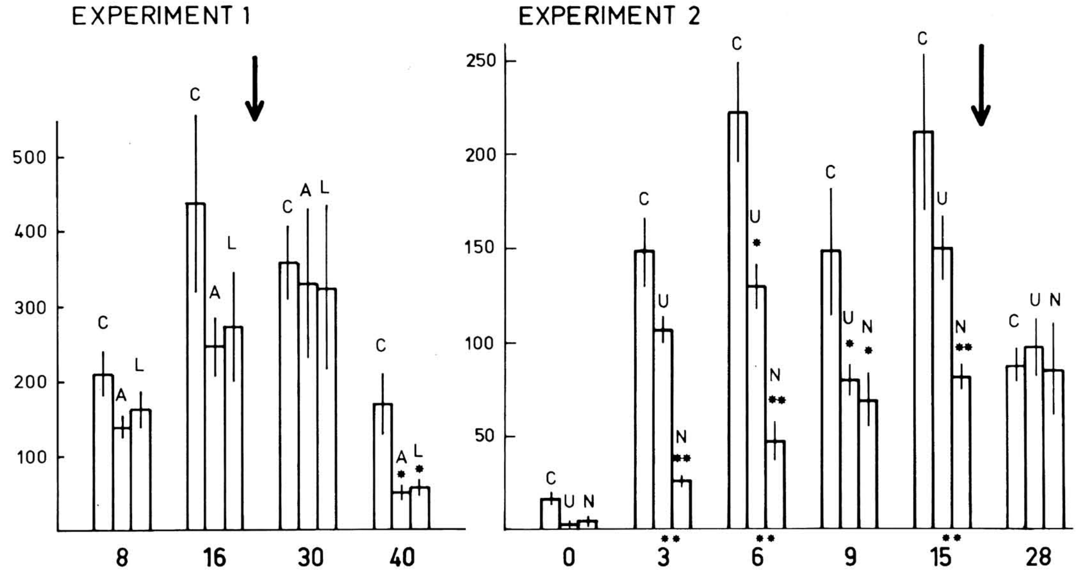

Fig. 1. Total numbers of Collembola per sample unit ±SE in the laboratory experiments. Significant differences between the treatments and the respective controls are indicated above the columns, the differences between U and N under the columns. The vertical arrows indicate between which samples the artificial winter was brought about. C = control, A = ash, L = lime, U = urea, N = NH_4NO_3. Numbers below the columns are weeks from the start of experiment.

pygmaea, *M. krausbaueri* and *M. minimus*. The effects of liming in Exp. 2 will be treated in Chapter 3.3.

Few significant effects of urea fertilization were recorded (Table 2), despite the double dosage used in the experiment. *W. anophthalma* and *M. pygmaea* were found in smaller numbers in all samples, *I. notabilis* and *M. minimus* in the first sample only. The difference in total numbers was not significant between urea treatment and the control, while it was significant in comparison with ash treatment, where the numbers were somewhat higher than in the control.

3.2. Laboratory experiments

The mechanical treatment during the establishment of the laboratory experiments destroyed most Collembola (see the first sample in Exp. 2, Table 3), but the populations recovered rapidly. The effects of the fertilizers were profound in comparison with those in the field experiments, even though the amount of chemicals applied was smaller when calculated per unit area.

In Exp. 1, all common species except *I. minor* showed significant differences between ash-treatment and the control, at least in one sample (Table 3). Both positive (*O. absoloni* and *I. notabilis*) and negative (*M. pygmaea*, *M. yosii* and *F. fimetarioides*) responses were recorded. The total numbers differed significantly in the last sample only (Fig. 1).

Urea (Exp. 2) considerably reduced both the total numbers (Fig. 1) and those of most species (Table 3), with the exception of the last sample. *I. notabilis* was the only species to show no response at all. *I. minor* recovered rapidly from an initial decrease, as did *F. fimetarioides*, though more gradually (Table 3).

3.3. The role of pH

Liming in field experiment 2 and laboratory experiment 1 was designed to result in the same soil pH as did fertilization with ashes. (Calcium, although itself a nutrient, is not considered to be a limiting factor.) This goal was reached satisfactorily (Table 4). No significant differences were observed between these two treatments for any species in either experiment (Tables 2 and 3, Fig. 1).

Table 4. Results of pH measurements. Each figure is a mean of several recordings. For the lab. experiments, values at the first and last samplings are given. See Huhta (1984) for details.

Field Exp. 1				
(Tammela)	Control	Ash	Ash+P	
0–3 cm	4.3	6.0	5.5	
3–6 cm	4.2	5.3	5.1	
Field Exp. 2				
(Ruotsinkylä)	Control	Ash	Lime	Urea
0–3 cm	4.4	7.0	7.2	5.5
3–6 cm	4.5	5.1	5.0	5.0
Lab. Exp. 1	Control	Ash	Lime	
	4.5, 4.8	6.9, 5.4	7.0, 5.5	
Lab. Exp. 2	Control	Urea	NH_4NO_3	
	4.4, 4.9	6.5, 6.1	3.8, 5.0	

In the second laboratory experiment, the same amount of nitrogen was given in two forms, urea resulting in a rapid increase in pH, and NH_4NO_3 causing a slightly acid initial reaction. It appeared that ammonium nitrate was toxic to Collembola, as it was to most other groups (see Huhta et al. 1983, 1984, Huhta 1984). The populations recovered slowly, but within about two months the total numbers attained the level of urea treatment (Fig. 1). *F. fimetarioides* and *I. minor*, the two species that finally exceeded the control level in the urea treatment, "lagged behind" in their recovery after NH_4NO_3 treatment (Table 3). By December all the differences had virtually disappeared.

4. Discussion

The interpretation of our results is not easy for two reasons. Firstly, the soil conditions at Tammela, where the experimental design was mainly aimed at monitoring tree growth after fertilization, proved to be rather heterogeneous, in spite of the apparent homogeneity of the stand. There were some significant differences in the densities of Collembola between the two control plots, and because there was only one replicate of the treatments ash and ash with P, these two should in fact be regarded as duplicates of the same treatment. (The slowly soluble phosphorus present in apatite hardly influenced the overall picture.) Secondly, there were only a few abundant species in common with Exp. 2, in which the design was more appropriate from the statistical point of view.

As a result of the first fact mentioned above, the differences between treatments and the control should be significant and of the same general nature in ashes and ashes with P, in order to be considered reliable. This holds true for *O. absoloni*, *F. quadrioculata*, *I. minor* and *I. notabilis*. Of these only *I. minor* and *I. notabilis* were present in reasonable numbers in common with the experimental site at Ruotsinkylä. *I. minor* showed some increase after liming in Exp. 2, while the reaction was the opposite in Exp. 1. *I. notabilis* was the only species to react similarly in both experiments. In addition, *W. anophthalma* showed some decrease after liming and *M. pygmaea* after urea treatment.

The soil for the laboratory experiments was taken from the same site as for field Exp. 2. The effects of different treatments were appreciably more marked than they were in the field. Most species present in sufficient numbers reacted significantly, either positively or negatively. In cases where comparison is possible, the reaction in the laboratory was of a similar nature to that in the field. This was also the case with *O. absoloni* and *I. notabilis*. In addition, *M. pygmaea*, *M. yosii* (*M. krausbaueri* s.l.), and *F. fimetarioides* decreased following all treatments.

There were no significant differences between liming and fertilization with ashes (Field Exp. 1, Lab. Exp. 2). Thus, as long as nitrogen is not included in the fertilizer, the results obtained can be explained by the change in pH rather than by the addition of nutrients. Hågvar & Abrahamsen 1980, 1984, Hågvar & Kjøndal 1981, and Hågvar 1984a, b have made thorough studies on the effects of acidification and liming on Collembola. Unfortunately, there are only a few species in common with our data to allow of comparison. There are obvious similarities between their results and ours, especially concerning the laboratory experiments. *M. pygmaea* and *M. yosii* reacted negatively, and *I. notabilis* positively, to the increase in pH. This is also in accordance with the observations by Bååth et al. (1980) and Heungens & van Daele (1984). With regard to *W. anophthalma*, *I. minor* and *M. minimus*, the results are not easily comparable. Melecis (1985) reported that the population densities of most collembolan species were lower in soil heavily polluted by a cement factory, which had resulted in a rise in the pH. However, in areas of moderate pollution, *F. mirabilis* and *F. quadrioculata* had increased their densities. (Heavy metals also contributed to the pollution in this case.)

The effect of urea on populations of Collembola was small in the field in spite of the double dosage used in Exp. 2. This is in accordance with the results of Axelsson et al. (1973) and Lohm et al. (1977), who could not observe significant changes when nitrogen fertilizers were applied in quantities used in practical forestry. However, when the dosage was increased essentially above the "normal" level, many species, as well as the total numbers, of Collembola exhibited a negative response, especially following fertilization with NH_4NO_3. Species in common with our data include *M. krausbaueri* s.l. and *I. minor*, which showed a similar response in our experiments, at least in the laboratory. The same holds true for the total numbers of Collembola as well.

Most species that exhibited a significant response to the ash and lime treatments in the laboratory responded similarly to the nitrogen fertilizers. Concerning urea, the explanation may lie in the rise in pH, while the toxicity of ammonium nitrate to Collembola obscures the interpretation with respect to N as a nutrient. The role of nitrogen as an agent causing the observed changes will be discussed in a later paper (Koskenniemi & Huhta in prep.).

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