The impact of moisture and temperature on adenylate content and adenylate energy charge of microbial communities in the litter of coniferous forest soils

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Synopsis

The impact of moisture and temperature on microbial populations in the litter of a spruce and two pine sites was investigated. The sites were located at different climatic regions: in Solling (Germany, high precipitation, coldest site), in central France near Foljuif (low precipitation, medium temperature), and in a coastal region near Wekerom (The Netherlands, medium precipitation, warmest site). The litter was equilibrated at different water contents ranging from 24% to 206% and was stored at different temperatures (2°C, 7°C, 14°C, 22°C). Adenylate content and Adenylate Energy Charge (AEC) were measured after one week of incubation.

Adenylate content in the coastal litter indicated an increased sensitivity of the microbial populations to low temperature (2°C) whilst in turn adenylate content in litter from the site with lowest rainfall indicated a decreased sensitivity to decreasing water content. Under extreme dry conditons (≤ 5% water holding capacity) the AEC below 0.4 in the litter from Wekerom pointed out that the cells were senescent. However, the other AEC values above 0.6 in the samples of all locations indicated microbial populations in the stationary or growing phase. Therefore it can be concluded that the microbial populations in the litter of the investigated sites were well adapted to a wide range of temperature and moisture conditions.

Adenylate content, Adenylate Energy Charge, moisture, temperature, microbial populations, forest soils

Synopsis

Der Einfluß der Feuchte und der Temperatur auf die mikrobiellen Populationen in der Streu von einem Fichten und zwei Kiefernstandorten wurden untersucht. Die Standorte lagen in verschiedenen klimatischen Regionen: im Solling (Deutschland, hohe Niederschläge, kühlste Fläche), in Zentralfrankreich nahe Foljuif (geringe Niederschläge, mittlere Temperatur) und in einer Küstenregion bei Wekerom (Niederlande, mittlere Niederschläge, wärmste Fläche). Die Streu wurde auf verschiedene Wassergehalte (24% bis 206%) eingestellt und bei verschiedenen Temperaturen (2°C, 7°C, 14°C, 22°C) inkubiert. Adenylatgehalte und Adenylat Energy Charge (AEC) in der Streu wurden nach einer Woche Inkubation gemessen.

Die Adenylatgehalte in der Streu der küstennahen Region deuteten auf eine erhöhte Empfindlichkeit der mikrobiellen Populationen gegenüber einer niedrigen Temperatur (2°C) hin, während umgekehrt die Adenylatgehalte von der Fläche mit dem geringsten Niederschlag (Foljuif) eine geringe Empfindlichkeit gegenüber sinkenden Wassergehalten hinwiesen. Unter extrem trockenen Bedingungen (≤ 5% der maximalen Wasserhaltekapazität) deutete der AEC unter 0,4 in der Streu aus Wekerom eine absterbende Population an. Die übrigen AEC-Werte lagen aber in den Proben aller Standorte über 0,6 und deuteten damit auf ruhende oder wachsende Populationen hin. Daraus kann geschlossen werden, daß die mikrobiellen Populationen in der Streu der untersuchten Standorte gut an ein breites Temperatur- und Feuchtespektrum angepaßt waren.

Adenylatgehalt, Adenylat Energy Charge, Feuchte, Temperatur, mikrobielle Populationen, Waldböden

1 Introduction

ATP detection is widely used as mass for microbial biomass and as an indicator for ecosystem perturbations (MEEGAN & al. 1996, NASEBY & LYNCH 1997, SHIBAHARA & INUBUSHI, 1997). But, some authors indicated that ATP may depend on physiological state of the microorganisms and others discussed that the sum of the adenylate content (AMP, ADP, ATP) may be a more reliable parameter to detect microbial biomass than ATP alone (DYCKMANS & RAUBUCH 1997, TSAI & al. 1997). Moreover, the use to describe the physiological condition of a given microbial population by the three components was demonstrated BROOKES & al. (1987). BROOKES & al. (1987) summarized that the concept of adenylate energy charge (AEC = {ATP+0.5 ADP} / {AMP + ADP +ATP}) was proposed to quantify the metabolic energy held in the adenine nucleotide pool and thus identify the state of a given microbial population: 0.8–0.95 growth conditions, 0.5–0.75 stationary phase and below 0.5 senescent cells. The AEC and the sum of

adenylates were used as physiological parameters to describe the relative fitness and size of the populations. Both parameters, sum of adenylates and AEC are used in the present study to describe the impact of environmental factors on microbial populations. In earlier investigations several authors stated that ATP-content fluctuate *in situ* because it depends on air drying, freeze drying, nutrient and moisture conditions AHMED & al. 1982, EILAND 1985, NANNIPIERI & al. 1990).

This study was carried out to investigate the combined impact of temperature and moisture conditions on the physiological state and size of microbial populations in the litter from coniferous forests. The study was based on the hypothesis that microbial communities which are adapted to different climatic conditions will differ in their sensitivity to water content and temperature.

2 Material and Methods

2.1 Sample preparation

Needles in the L-layers of pine stands were cutted using scissors. All materials from L-layers were homogenized by hand mixing. Actual water content (WC) was estimated using the mixed materials. For estimation of maximum water-holding capacity (WHC) glass tubes with a porous quarz bottom (10 cm height and 2 cm diameter) were half filled with the field fresh sample. The weight of the glass tubes and of the fresh material was noted. The glass tubes were covered and incubated over 12 hours in a water bath. After twelve hours the tubes were put on wet filter paper for half an hour in order to avoid an overestimation. Then the weight of the glass tube with the water saturated sample was noted. This procedure was repeated three times with shortened incubation periods (two hours) to control the weight of the water saturated sample. Finally the sample was dried over 12 hours at 105°C. WC and WHC were calculated as the relative (%) of the dry weight.

2.2 Detection of Adenylates and Adenylate Energy Charge (AEC)

Measurements of adenine nucleotides and calculations of the adenylate energy charge (AEC) were made according to the procedure of BAY & al. (1989). Dimethylsulfoxid (DMSO), Na_3PO_4-buffer (10mM) and NRB (Nucleotide Releasing Reagent for microbial ATP, Lumac, The Netherlands) were used as extractants. After derivatization with chloracetaldehyde, the adenine nucleotides were determined by HPLC with fluorescence detection. The given values are the sum of the adenylates AMP, ADP and ATP.

All presented values are arithmetic means and standard deviations.

2.3 Experimental design

In order to study the combined effect of temperature and moisture conditions an additional method had to be employed. Needles were collected in the L-layer of three coniferous forest stands. Two of sites, Wekerom and Foljuif were pine stands (*Pinus sylvestris spec.*) and Solling site was a spruce stand (*Picea abies spec.*) (Table 1).

Maximum water-holding capacity of the litter averaged out to have a water content of 500%. Before the experiment it was checked that the microbial activity with a water content of approx. 200% was still at its maximum. After installing the moisture contents (intended moisture contents 15% {Wekerom litter only}, 50%, 100%, 150% and 200%) the litter-samples (n=5) were stored for seven days at 2°C, 7°C, 14°C, and 22°C in glass vials (100 ml) covered by polyethylen foil. After seven days the samples were removed from the storage room and microorganisms killed with DMSO, thereby stopping biological activity immediatly. This procedure guaranted that the actual physiological state of the microbial population was detected.

3 Results

As mentioned before the litter was collected from sites with different climatic regime. The coastal site near Wekerom had mediate precipitation and highest annual mean temperature (Table 1). The continental sites represented two extremes. Solling had lowest annual mean temperature and Foljuif had lowest precipitation (Table 1). In the experiment it was investigated whether the populations showed specific reactions with regard to manipulated water content (WC) and temperature. The water content at the x axsis represents an average value of all samples at this moisture level and includes therefore the moisture values of the samples at all four temperatures.

In general, adenylate content in Solling litter was on the highest level but, there were few combined effects of temperature and water content on adenylate content (figure 1). Adenylate content in the litter from the coastal site near Wekerom tended to decraese at low temperature (2°C) if water content was high and not a limiting factor (figure 1). But, the impact of temperature on adenylate content was low at the continental sites Solling and Flojuif. On the other hand, decreasing water content influenced adenylate content in the litter from Wekerom and Solling negatively. Adenylate content in litter from Foljuif, the

Table 1
Description of the sampling sites and characterisation of the litter.

Tab. 1
Beschreibung der Probeflächen und Charakterisierung der Streu.

Description of the sampling sites									
Site	Country	Longitude	Latitude	Elevation m	Precipitation* mm	Temperature* °C	Stand	Age (yrs)	Soil texture
Wekerom	The Netherlands	52°1'N	5°4'E	23	720	13.0	*Pinus sylvestris*	35	loamy sand
Foljuif	France	48°2'N	2°4'E	83	559	11.0	*Pinus sylvestris*	30	loamy sand
Solling	Germany	51°8N	9°5'	500	968	6.4	*Picea abies*	109	silt loam /silty clay loam

Litter characteristics								
Site	pH ($CaCl_2$)	C % DW	N % DW	P mg g DW^{-1}	C/N ratio	C/P ratio	Cmic µg g DW^{-1}	microbial activity (µW g DW^{-1})
Wekerom	3.9	47.7	1.5	0.62	32.9	785	6689	606
Foljuif	3.8	40.9	1.3	0.81	32.2	519	7067	697
Solling	3.5	43.9	1.6	1.03	27.4	447	8713	934

* annual mean

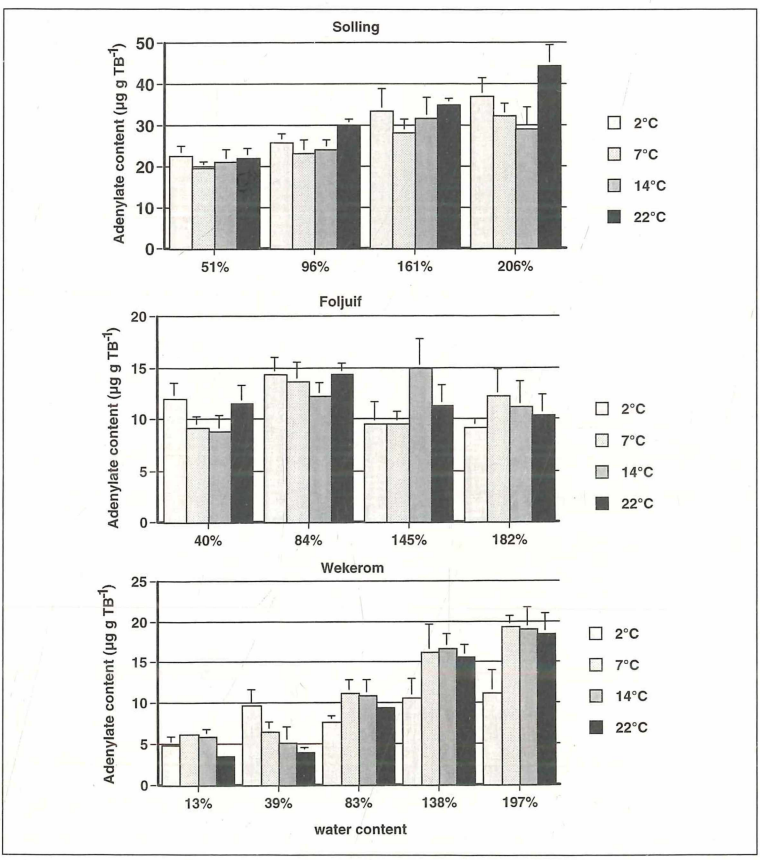

Fig. 1
Adenylate contents (∑ AMP, ADP, ATP µg g DW^{-1}) in the L layers of three coniferous forest stands one week after incubation at different temperature and moisture conditions (n = 5).

Abb.1
Adenylat Gehalt (∑ AMP, ADP, ATP µg g DW^{-1}) in den L-Auflagen der drei Nadelwälder nach einer Woche Inkubation bei unterschiedlichen Temperatur- und Feuchtebedingungen (n = 5).

site with lowest throughfall input was not affected by water content below 50%.

However, the adenylate energy charge indicated that the given populations were well adapted, since the AEC ranged between 0.5 and 0.75 (stationary phase) and above 0.8 (growth phase) (figure 2). Only at the extremly low water content level of 13% (Wekerom litter) did the AEC decrease below 0.5 (senescent cells). The extremly low AEC at 39% WC of Wekerom litter at 22°C has to be discussed as an artefact. The low value can be explained by the extremely dry water content (24%). It seems unlikely that temperature caused the decrease.

4 Discussion

From the results presented here it can be concluded that temperature did not to seem to influence the adenylate content in two of three sites. This observation agrees with the findings of PÖHACKER & ZECH (1995), who reported that microbial biomass Cmic was not influenced by incubation experiments at different temperatures. But, the decrease of adenalytes in the litter of the coastal site Wekerom at conditions of low temperature (2°C) and high moisture indicates a combined effect of both factors on the microbial population. A special sensitivity of the microbial population may be assumed to low temperature if moisture conditions are not limiting. As a possible interpretation it may be assumed that the microbial population from the coastal region was adapted to moderate temperature conditions.

CIARDI & al. (1993) investigated microbial populations in agricultural soils and showed that the sum of adenylates did not decrease after air drying. This is in accordance to the findings in the present study in the litter from Foljuif site. But, in difference to the findings of CIARDI & al. (1993) the sum of adenylates decreased in the two sites with high annual precipitation Wekerom and Solling. This may be interpreted that the microbial populations in the litter from the two sites were more sensitive to moisture than the population in the litter from Foljuif site. A possible explanation could be that the microbial populations in the different litter layers were adapted to different moisture conditions.

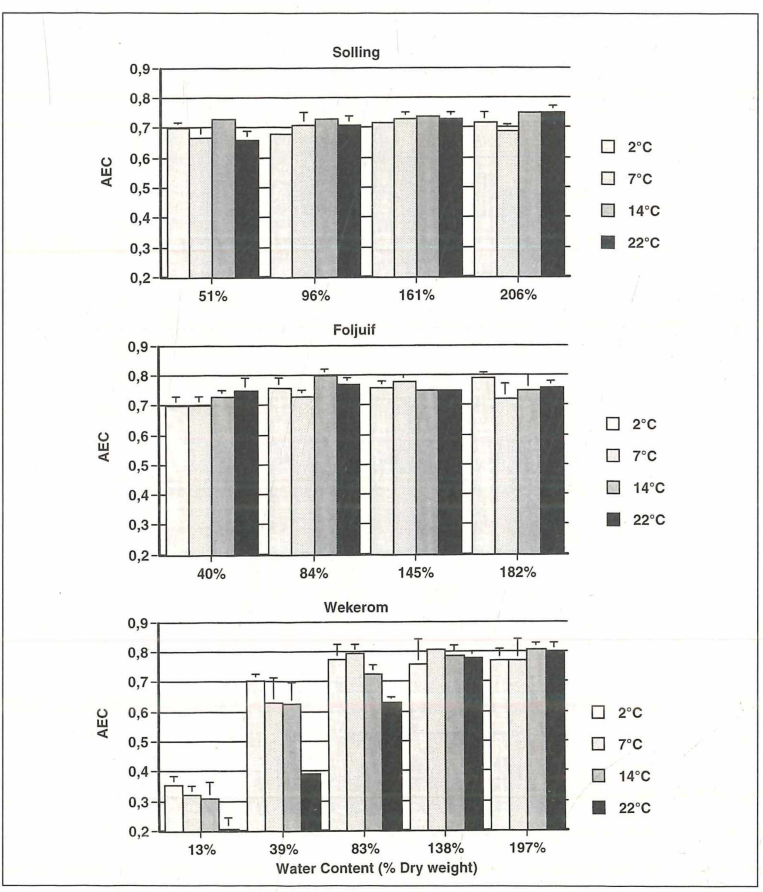

Fig. 2
Adenylate Energy Charge (AEC) in the L layers of three coniferous forest stands one week after incubation at different temperature and moisture conditions (n=5).

Abb. 2
Adenylat Energy Charge (AEC) in den L-Auflagen der drei Nadelwälder nach einer Woche Inkubation bei unterschiedlichen Temperatur- und Feuchtebedingungen (n=5).

Some publications reported that AEC in soils will decrease during drying reported of AEC values of 0.46 and 0.5–0.57 respectively (BROOKES & al. 1983, ROSACKER & KIEFT 1990). CIARDI & al. (1993) showed also that ATP may drop and AMP and ADP may increase if agricultural mineral soils were air dried down to 5% of waterholding capacity. But they found AEC values below 0.4. Our results of AEC in the litter from Wekerom dropped down below 0.4 at same moisture conditions and were in accordance to the findings of CIARDI & al. (1993). However, the AEC above a water content of 25% (5% of water holding capacity) indicated that the microbial biomass maintained at metabolic energy levels which were interpreted by BROOKES & al. (1987) to be characteristic for organisms in stationary phase up to growing phase. Therefore, it can be concluded that the microbial populations in the litter from all sites were tolerant to a wide range of temperature and moisture conditions.

5 Conclusion

Adenylate content in the present study indicated specific adaptions of local populations to specific temperature and moisture conditions. But, in general, the AEC values in the litter from the three sites can be interpreted such, that the microbial populations are in general well adapted to wide ranges of moisture and temperature conditions.

6 Acknowledgements

I thank Prof. Dr. Beese for discussion and support. This work was done in the Institute for Soil Ecology, GSF, Munich. I thank the BMBF, E.S.F. and CEC (STEP-CT91-0118) for funding and the members of CORE-project for the cooperation. I thank Mrs. Angelika Schulz for reliable technical assistance.

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