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Higher Vulnerability of Heterotrophic Soil Respiration to Temperature Drop in Fallows than in Meadows

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Abstract: The present study examines the difference between stability and climatic factors of soil microbial communities in two ecosystem types with similar plant biomass, while differing in plant diversity, successional stage and complexity. Observations of variation in stability can be applied to climate change investigations, a topic of current pivotal importance.

We compared responses of soil basal respiration to short time perturbation in soils collected from six established (meadow) and six early successional (fallow) ecosystems by exposing them to thermal and water stress. Resistance and resilience indices were calculated to describe how much a system was affected by and recovered from perturbation. The soil's physico-chemical properties and plant community composition were identified and used for correlation and regression analyses with the stability indices.

There was a smaller relative change in soil respiration in meadows than in fallows as temperature decreased from 22 to 10°C. Resistance to coolness was correlated to higher soil pH, while resilience to plant species richness. The drying-rewetting experiment highlighted that the stability indices became non-linear when a data set had high variations.

Soil microbial communities in a more complex and mature ecosystem type (meadow) were more stable under a moderate perturbation. This might have been supported by co-occurring factors, with soil pH being the most influential. The slightly acidic fallow soil might have a higher potential for carbon sequestration than neutral meadow soil.

Keywords: Climatic factors; Meadow; Fallow; Resistance and resilience indices; Stability; Temperature fluctuation.

Introduction

Historically, drought, rainfall and temperature fluctuations were studied in laboratory experiments to reveal functional ecology aspects such as the resistance and resilience of ecosystem communities. Observations of variation in ecosystem stability can also be applied to climate change investigations, a topic of pivotal importance, as the Earth's atmosphere warming is increasingly evident (https://climate.nasa.gov/; Lenssen et al., 2019). Yet, the estimation and modelling of its effect on a wide range of elements that can be affected, including biogeochemical processes, among others, are a challenge. Laboratory experiments can be helpful in the assessment of particular causes of climate changes by measuring end-points in modified conditions. Effects of drying and rewetting on ecosystem stability have been described, for example, by Orwin and Wardle (2004), Orwin et al. (2006) and White et al. (2012). These experimental studies varied in design and factors tested (White et al., 2012) and used different indices of resilience (Orwin and Wardle, 2004, Griffiths and Philipotte, 2013). Their results were largely mixed and complex so that a broad understanding of ecosystem

response to climatic disturbances remained elusive (White et al., 2012). The response of soil respiration to temperature has been studied chiefly in the context of climate change, resulting in a number of meta-analyses and reviews (e.g., Davidson and Janssens, 2006; Bond-Lamberty and Thomson, 2010; Hursh et al., 2017; Zhao et al., 2017). A positive relationship between CO_2 efflux and the two microbial activity modulators, temperature and humidity, has been confirmed for most of the biomes (Hursh et al., 2017; Zhao et al., 2017). Variable interactions between nitrogen deposition and climate warming have also been reported (Balser et al., 2010; Liu et al., 2016). Because decomposition could be more temperature-sensitive than net primary production (Kirschbaum, 2000; Lloyd and Taylor, 1994), the response of soil respiration to temperature is a critical link between climate change and the global C-cycle (Allen et al., 2011). On the other hand, the evolution of C-cycling traits in response to climate change could accelerate or decelerate the current trend in rising atmospheric CO_2 (Monroe et al., 2018).

Soil basal respiration is one of the biological indicators suggested for monitoring soil health in the light of climate change (Allen et al., 2011). While numerous studies have examined the effect of temperature rise on soil heterotrophic respiration, low-temperature respiration has been neglected. The available literature mainly examines frost conditions, leaving a gap in moderately low temperatures, ranging from 0ºC to 20ºC, while, as a result of climate change, a decrease in the number of winter days with extremely low temperatures is predicted (IPCC, 1996) and increasing mean temperature of winters has been observed in Europe (Luterbacher et al., 2004).

Here, we addressed the question: Do soil microbial communities inhabiting young and less complex ecosystems undergoing succession changes (fallows) differ in resistance and resilience to climatic disturbances from more constant and complex ecosystems (meadows)? By complexity, we understand greater species diversity of vascular plants, with a higher diversity intrinsically involving a higher number of interspecific interactions. As explained by Liu (2004), "The concept of ecological complexity stresses the richness of ecological systems and their capacity for adaptation and self-organization." We use constancy sensu dynamical stability, to refer to ecosystems that are not under succession, i.e., changing little over time. By resistance, we understand "the capability of a system to remain unchanged in the face of external pressures such as disturbances" (Scherer-Lorenzen, 2005), while resilience is defined as "the ability of a system to return to its original or equilibrium state after it has been displaced from it by external pressures" (Scherer-Lorenzen, 2005). Traditionally, the stability of an ecosystem is its ability to return to its initial state following a disturbance (May, 1972, 1973 in Berendse, 1994; Pimm, 1984). Often, stability is reduced to the sum of resistance and resilience (e.g. Griffiths et al., 2001; Orwin et al., 2006). We aimed at exploring the two ecosystem stability indices from a traditional angle (e.g. Pimm, 1984; Berendse, 1994; McNaughton, 1994; Botton, 2006; Ives and Carpenter, 2007), in the context of the topical issue of ecosystem response to climatic factors.

To investigate our question, we measured the effects of temperature and humidity on fallow and meadow soil microbial community's respiration and calculated resistance and resilience indices. Fallows and meadows differ substantially by biological complexity and stability. Fallows, i.e., abandoned farmlands, are young ecosystems in the initial stage of secondary succession. Plant communities of fallows were typical R-selected communities with strong domination of competitive weed and ruderal species (in particular *Elymus repens,* followed by *Utrica doica, Cirsium arvense* and *Chaerophylum aromaticum;* Chmolowska et al., 2016, 2019), which grow in a wide range of conditions and rapidly overtake bare lands. Meadows, if managed sustainably and systematically over a long time, are relatively more constant and longer lasting ecosystems than fallows. Even though meadows need to be mown or grazed, if they are not to be overgrown by bush and forest, they accumulate a larger number of plant species during their long time of existence, resulting in the dense, well-developed root system and high diversity, which assigned them to more saturated K-selected communities than fallows. Meadows showed mutualistic relationships with microbial communities, which confirmed their high level of self-organisation (Chmolowska et al., 2016, 2017). In particular, meadows maintained a high plant diversity in nutrient-depleted conditions, supported by the higher contribution of legumes delivering nitrogen and roots' colonisation by mycorrhiza, which most probably provided phosphorus and potassium. The fresh meadows chosen for the research represent communities of the *Arrhenatherion* alliance. They are protected by the Natura 2000 network as a valuable, endangered, and rapidly *disappearing habitat* type in Europe. Traditionally managed meadows are biodiversity reservoirs (Plieninger et al., 2006).

This study is a part of a larger project, in which it has been shown that even though two semi-natural grassland

types are located in the same area, they differ in their soil properties due to presumed remains of fertilisers on ex-arable fields and humus accumulation in the older ecosystem of the meadow (Chmolowska et al., 2016). The biomass, cell numbers, and structure of the soil prokaryotes and micro-eukaryotes were mostly similar between the two ecosystem types, except arbuscular mycorrhiza, which was more frequent in roots in meadows compared to fallows (Chmolowska et al., 2017a). Significant differences between meadows and fallows were observed in soil processes. Respiration, cellulose decomposition, and nitrogen mineralisation rate confirmed microbial functional niches as dependent on the ecosystem type (Chmolowska et al., 2017a, 2017b). These results were obtained from experiments on soil incubated in normalised and constant conditions of temperature (22ºC) and moisture (70% air humidity, 55% soil Water Holding Capacity, WHC). In this study, we tested the response of the soil microbial community in fallows and meadows to disturbed climatic conditions.

Methods

Study Plots

Six fallows and six meadows were chosen for this research located in the Beskid Sądecki Mountains (Polish Western Outer Carpathians), near the town of Krynica-Zdrój (49º 25′ 17″ N; 20º 57′ 33″ E). The climatic conditions were as: vegetation period 170-185 days; annual average precipitation 890 mm; average annual temperature 4ºC above 600 m a.s.l. (fallow 1, 6; meadow 1, 2, 3) and 6ºC at the range 500-600 m a.s.l. (all remaining plots). The climate is humid continental (https://pl.climate-data.org/europa/polska/lesser-poland-voivodeship/krynica-zdroj-29495/). Fallows were characterised by the presence of ruderal and segetal weeds (especially couch grass *Elymus repens*) and low plant diversity: less than 27 species per 25 m^2 plot (Table 1). Meadows had high plant diversity (35-50 plant species per 25 m^2 plot) and a high proportion of species characteristic for the *Molinio-Arrhenatheretea* class (Chmolowska et al., 2016). The soil in fallows had higher contents of available P and K, and NO_3^- than meadow soils and a slightly lower C:N ratio and WHC (Chmolowska et al., 2016). Fallows and meadows had statistically similar soil pH; however, fallows consisted of acidic and slightly acidic soils, while the meadow soils ranged from acidic to alkaline (Table 1; Chmolowska et al., 2016).

For the climatic fluctuations experiment 10 soil cores of 0-10 cm deep were taken from each site along the 20 m transects in June 2010. Cores from one plot were pooled and then sieved through 4 mm mesh. Field moist soils were stored at 4ºC before the examinations. Organic matter content, soil pH in 1M KCl and water, and WHC were examined during this sampling campaign. Other soil physico-chemical and biological properties and vegetation composition were known from the previous studies (Chmolowska et al., 2016, 2017a, 2017b); raw data is available in a database (Chmolowska, 2019).

The following experiments were performed in climate chambers with regulated temperature and humidity in July 2010.

Respiration under Warming-Cooling

Six fallow and six meadow field moist soil samples, corresponding to 10 g dry weight (dwt), were put into 100 ml respirometry bottles, preincubated for three days in normalised conditions (22ºC), watered to 55% WHC a day before the experiment, and screwed into the Micro-Oxymax respirometer (Columbus Instruments) located in the climate chamber. The samples were then exposed to temperature changes using the four following temperature steps, each lasting for 24 hours: 22ºC, 30ºC, 22ºC and 10ºC. After the cycle completion, the samples were maintained at 22ºC for three days (Figure 1). The temperature changes were automatically set by the programmed climate chamber and additionally confirmed by the Micro-Oxymax respirometer, which records the temperature during each reading. This experiment layout was based on work by Orwin and Wardle (2006) with the changing of drying-rewetting disturbance to warming-cooling disturbance. The control soil herein was the soil before any treatments (t_0). The respiration rate was measured automatically in 2 h 15 min intervals during seven days of the experiment (Figure 1). Because approximately four hours were needed for the temperature in the incubation chamber to reach the target level, raw data were screened for the respiration records taken while adjusting the temperature. In this way, the first respiration measurement series from each temperature change was discarded because the incubation chamber did not reach the target temperature by then. Thus, only records taken at constant temperatures (22ºC, 30ºC and 10ºC) were used to calculate mean temperature-specific respiration rates and conduct statistical analyses.

To compare the magnitude of the respiration response to temperature change in the soil samples, respiration rates at 30ºC and 10ºC were expressed as a percent of the respective respiration rates at 22ºC preceding

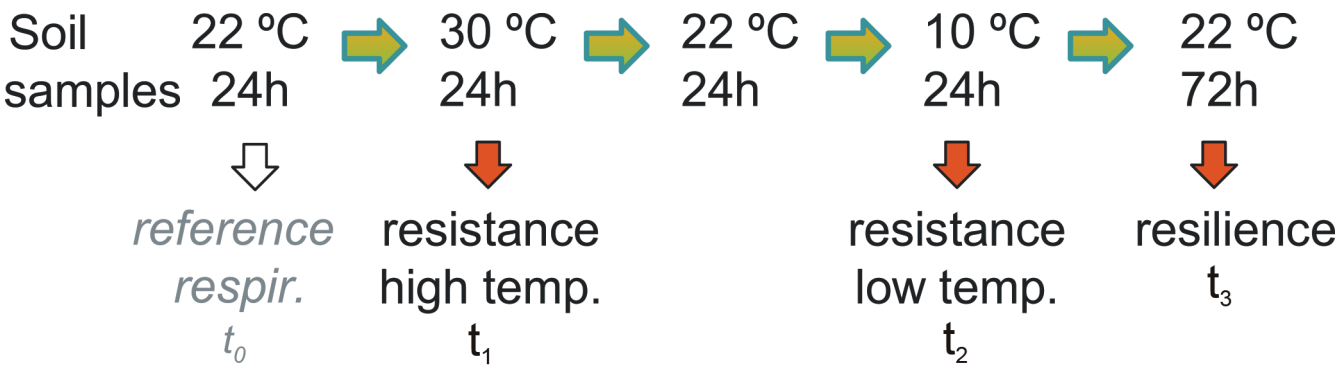

Figure 1: Scheme of the warming-cooling experiment.

each 30ºC or 10ºC period, respectively. Resilience was calculated from the mean respiration rate for three days of stabilisation in 22ºC (t_3) as a percent of respiration measured at the beginning of the experiment, before any temperature changes: (t_3/t_0) ·100. In addition, resistance and resilience were calculated using indices proposed by Orwin and Wardle (2004). Resistance = $1 - (2\cdot|D_0|)/(C_0+|D_0|)$, where D_0 is the difference in the respiration rates between the control (C_0) and the disturbed soil after the disturbance (t_1, t_2). Resilience = $(2\cdot|D_0|)/(|D_0|+|D_3|) - 1$, where D_3 is the difference between the control and the disturbed soil during three days of recovery (t_3).

Normal distribution of calculated resistance and resilience of fallows and meadows were confirmed with the Shapiro-Wilk W test in Statgraphics Centurion XVI.I (Statpoint) and were compared with the Student' *t* test in Microsoft Excel 2010. Each Student's *t* test was preceded with an *F* test to examine if variances of the two groups were homogenous. If heteroscedasticity was detected (*F* test $p < 0.05$), the heteroscedastic (heterogenous variance) option of the Student's *t* test was used. A correlation matrix was run in the Statgraphics Centurion programme, with resistance and resilience significantly different between the two types of ecosystems and to observe which properties might explain the observation. The variables were checked if they ranged within standardised skewness and kurtosis |2|, which informs about the normality of the distribution. Altitude, plant species number (richness) and the following soil chemical properties of the sites were used: K_2O, P_2O_5, N-NO_3 and N-NH_4; total C, N, Mn, Ca, and Na content; cation exchange capacity (data retrieved from Chmolowska et al., 2016; Table S1); organic matter content, Water Holding Capacity (WHC) and soil pH (determined in soils sampled in 2010) in a matrix (Table 1). Significant correlations were further tested with a linear regression in Statgraphics Centurion.

Respiration under Drying and Rewetting

In this experiment, the soils were exposed to changes in moisture. The procedure followed the study by Orwin

Table S1: Higher vulnerability of heterotrophic soil respiration to temperature drop in fallows than in meadows

	N [%]	*C [%]*	*Ca [mg/kg]*	*Na [mg/kg]*	*Mn [mg/kg]*	*CEC [cmol(+)/kg]*	*K_2O [mg/kg]*	*P_2O_5 [mg/kg]*	*N-NO_3 [ug/g]*	*N-NH_4 [ug/g]*
Fallow 1	0.25	2.52	242.27	88.74	962.82	1850.65	576.7	18.85	14.99	5.44
Fallow 2	0.20	2.05	157.55	51.06	444.82	1201.78	432.3	28.01	10.40	3.88
Fallow 3	0.24	2.50	207.91	60.70	627.60	1062.12	75.8	42.61	14.32	1.96
Fallow 4	0.25	2.56	112.64	61.79	303.31	930.77	107.0	15.32	6.38	3.34
Fallow 5	0.19	2.04	621.38	50.15	665.55	1736.63	238.5	71.04	12.29	3.34
Fallow 6	0.36	3.66	632.68	65.00	968.61	2365.40	270.5	74.67	15.65	1.68
Meadow 1	0.32	3.39	400.77	97.41	655.57	2611.34	84.0	0.62	2.49	2.31
Meadow 2	0.27	3.01	166.01	62.64	691.44	1327.96	98.5	6.83	0.58	7.04
Meadow 3	0.40	4.37	772.09	111.73	1218.08	5597.50	81.5	4.44	0.14	4.15
Meadow 4	0.24	2.56	177.45	61.10	452.38	941.85	86.0	3.58	0.00	4.31
Meadow 5	0.31	3.75	10164.39	88.10	453.94	3121.13	83.0	4.82	6.20	2.28
Meadow 6	0.23	2.48	1085.31	51.88	533.07	2111.47	121.3	8.26	0.52	1.38

et al. (2006). Field moist soil samples, one per each of the six fallows and six meadows, corresponding to 50 g dwt, were put into 1 L glass jars and preincubated for four days at 22ºC and 70% air humidity. On the fifth day, the samples were rewetted to 55% WHC. Control samples were incubated while covered, and the remaining samples were left open to dry overnight. Because the evaporation was slow, the samples were additionally dried in the morning with a fan until they reached 10-15% WHC. The jars were closed, and the evolved CO_2 was captured in 0.1 M NaOH for 24 hours (t_1). After 24 h, all samples were rewetted to 55% WHC, and all jars were closed again for the next 24 h for respiration measurement (t_2). Two days later, i.e., three days following rewetting, the respiration rate was measured again (t_3) (Figure 2). The respiration rate was measured by the titration method as described in Niklińska et al. (2005). The time-points t_1 and t_2 were used to calculate the resistance to drying and rewetting, respectively, while t_3 was used to calculate resilience. Resistance and resilience were calculated as described above, following Orwin and Wardle (2004), in addition to Orwin et al. (2006). Simultaneously, change relative to control, expressed as the percent of the respective respiration rates was calculated for t_1 (drying), t_2 (rewetting), and t_3 (recovery) for evaluation of the Orwin and Wardle (2004) indices.

Results

Effect of Warming-Cooling on Fallow and Meadow Soil Respiration

The basal respiration rates of meadow soils were higher than of fallows apart of a fallow with a higher content of organic matter (fallow 1; Table 1). The increase in incubation temperature from 22ºC (measured before any temperature change, t_0) to 30ºC increased the soil respiration rate by approximately 76.4-78.2%. A drop in temperature from 22ºC to 10ºC decreased the respiration rate by approximately 68.9-71.3% (Table 2). There was no difference between the two ecosystem types in response to temperature increase from 22ºC to 30ºC (Student's t test $p > 0.05$). In the temperature drop from 22ºC to 10ºC, respiration decreased relatively less in meadows than in fallows (t = -2.42; $p = 0.04$; Table 2). Resilience exhibited a similar pattern: meadow soils were observed to be more resilient than fallow soils (t = -2.37; $p = 0.04$; Table 2). Resistance to temperature drop and resilience was correlated ($r = 0.83$; $R^2 = 68.97\%$; $p < 0.001$). The correlation analysis of physico-chemical soil properties, altitude and plant richness pointed to only a correlation between resistance to the temperature decrease and soil pH ($r = 0.78$; $R^2 = 60.81$;, $p = 0.003$; Figure 3) and also in resilience and plant richness (r

Table 1: Properties of each fallow and meadow, means and standard deviations. Soil respiration is soil basal respiration before temperature fluctuations (t_0). Significant differences of Student's t test ($p < 0.05$) are in bold

	Altitude m asl	*OM %*	*pH [H_2O]*	*pH [KCl]*	*WHC g H_2O g^{-1}*	*Plant richness*	*Soil respiration ul CO_2 hr^{-1} g^{-1}*
Fallow 1	710	9.1	5.9	4.7	1.3	22	4.15
Fallow 2	580	5.0	5.5	4.6	0.7	13	1.57
Fallow 3	500	5.4	5.8	5.0	0.9	23	1.59
Fallow 4	530	6.4	5.5	4.7	1.0	26	2.47
Fallow 5	510	4.7	6.4	6.0	0.8	19	1.76
Fallow 6	640	6.6	6.1	5.5	0.9	16	2.22
Meadow 1	725	9.6	6.6	5.8	1.1	47	2.79
Meadow 2	730	8.0	5.5	4.3	1.0	42	3.29
Meadow 3	720	11.4	6.6	5.5	1.6	50	3.74
Meadow 4	580	6.2	5.5	4.5	0.9	41	2.59
Meadow 5	520	6.8	7.6	7.1	1.0	37	2.46
Meadow 6	560	5.7	6.9	6.4	0.9	46	2.7
Mean f ± SD	578 ± 83	6.2 ± 1.6	5.9 ± 0.4	5.1 ± 0.2	0.9 ± 0.2	**20 ± 5**	2.3 ± 1.0
Mean m ± SD	639 ± 96	7.9 ± 2.2	6.5 ± 0.8	5.6 ± 0.3	1.1 ± 0.2	**44 ± 5** ***	2.9 ± 0.5

Significance level: *** $p < 0.001$.

Table 2: Results of warming-cooling experiment. Relative respiration (percentage of control respiration t_0), and resistance and resilience indices (O–W index; Orwin and Wardle, 2004) for each fallow and meadow, means ± standard deviations. Significant differences of Student's *t* test ($p < 0.05$) are in bold

	Relative respiration at 30°C *[% t_0]*	*Relative respiration at 10°C* *[% t_0]*	*Resilience* *[% t_0]*	*Resistance30* *[O–W index]*	*Resistance10* *[O–W index]*	*Resilience* *[O–W index]*
Fallow 1	172.9	30.7	81.9	0.16	0.16	0.6
Fallow 2	177.7	26.8	65.3	0.13	0.13	0.37
Fallow 3	184.4	27.6	70.7	0.08	0.14	0.44
Fallow 4	170.9	29.7	73.3	0.17	0.15	0.47
Fallow 5	177.1	29	78.5	0.13	0.16	0.55
Fallow 6	175.2	28.4	71.5	0.14	0.14	0.45
Meadow 1	176.8	29.9	75.3	0.13	0.15	0.5
Meadow 2	171	30.5	78.9	0.17	0.16	0.55
Meadow 3	178.4	30.4	83.6	0.12	0.16	0.63
Meadow 4	177.7	29.2	79.8	0.13	0.15	0.57
Meadow 5	192.5	33.5	84.8	0.04	0.2	0.63
Meadow 6	173	33	79.8	0.16	0.17	0.56
Mean f ± SD	176.4 ± 4.68	**28.7 ± 1.41**	**73.5 ± 5.94**	0.13 ± 0.03	**0.15 ± 0.01**	**0.48 ± 0.08**
Mean m ± SD	178.2 ± 7.55	**31.1 ± 1.75** *	**80.4 ± 3.41** *	0.12 ± 0.05	**0.17 ± 0.02** *	**0.57 ± 0.05** *

Significance level: * $p < 0.05$.

= 0.62; R^2 = 38.09%; p = 0.03). Percentage changes in respiration correlated strongly with the calculated resistance and resilience indices, for warming: $r = -1$, p = 0.002, and for cooling: r = 0.91, p = 0.004.

Effect of Drying-Rewetting on Fallow and Meadow Soil Respiration

Drying decreased soil respiration rate by about 17%. Differences between fallows and meadows were

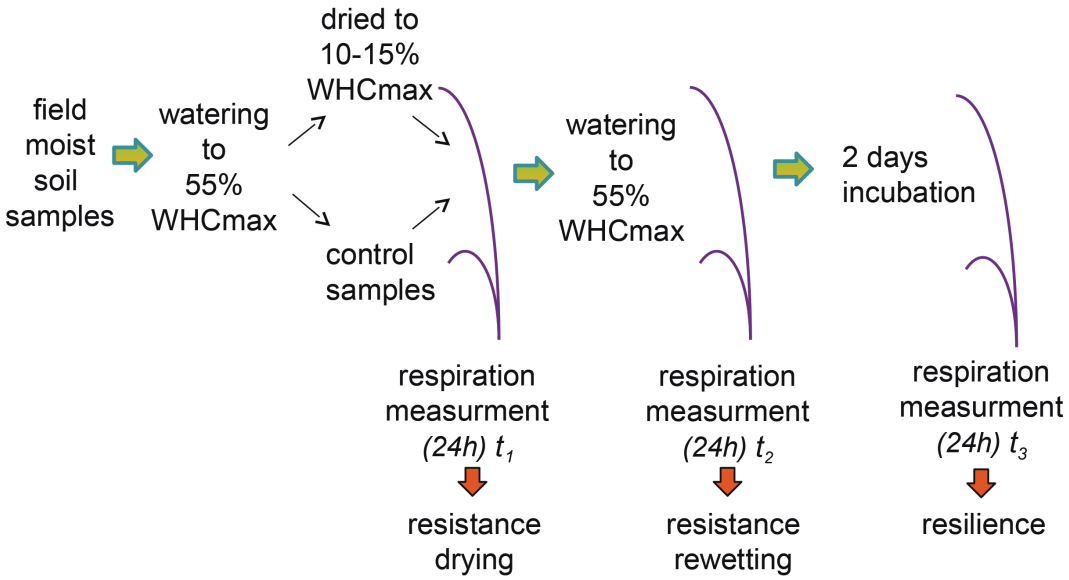

Figure 2: Scheme of the drying-rewetting experiment following Orwin et al. (2006).

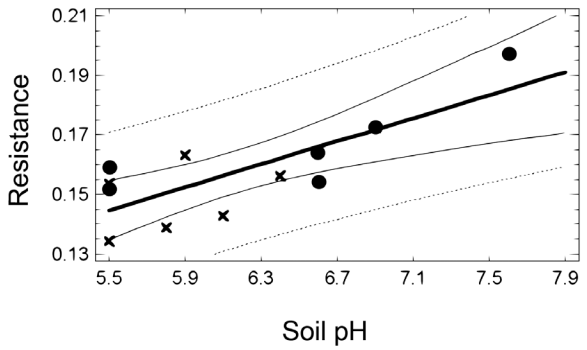

Figure 3: Linear regression with the scatterplot of the observations, the least squares regression line, the prediction bands (dark grey, bold line) and confidence interval bands (light grey line); filled circles indicate meadows, crosses indicate fallows. Resistance index to temperature drop = 0.03 + 0.02 · pH, r = 0.77, R^2 = 59.39%, p = 0.003.

observed neither in resistance to drying nor in rewetting and resilience (Table 3). Two fallow soils, 5 and 6, exhibited a strong negative resilience index to drying and rewetting, as the difference in respiration rates between control and the treatment was much higher after the recovery period than at the disturbance time, resulting in an extremely high standard deviation of resilience in fallow soils (Table 3). Consequently, while the calculated resistance index to drying correlated strongly to the respective respiration change (r = 0.99; p < 0.001) showing the very close similarity between the two ways of calculating the response effects; resilience did not (p >> 0.05), because of high variability and non-linearity of the resilience index. There were no significant correlations of soil chemical properties with a resistance to drying. We did not perform correlation analysis for resilience to drying and rewetting because these indices were non-linear.

Discussion

In this study, meadow soils were less responsive to temperature drop than fallows confirming their lesser sensitivity. Higher resistance and resilience, or just higher resistance of grassland soil in comparison to farm or disturbed soil were observed in other studies, which used a range of stressors (Degens et al., 2001; Griffiths et al., 2001; de Vries et al., 2012). We found that soil pH moderated microbial community sensitivity to cooling. The relationship between soil pH and microbial

Table 3: Results of drying and rewetting experiment. Relative respiration (percentage of control respiration) and resistance and resilience indices (O–W index; Orwin and Wardle, 2004) for each fallow and meadow, means ± standard deviations. There were no significant differences between fallows and meadows

	Relative respiration drying	*Relative respiration rewetting*	*Resilience*	*Resistance drying*	*Resistance rewetting*	*Resilience drying*	*Resilience rewetting*
	[% control]	*[% control]*	*[% control]*	*[O–W index]*	*[O–W index]*	*[O–W index]*	*[O–W index]*
Fallow 1	92.2	103	96	0.86	0.94	0.43	-0.08
Fallow 2	69.1	180.5	116.3	0.53	0.11	0.43	0.72
Fallow 3	74.3	54.4	83.8	0.59	0.37	0.39	0.71
Fallow 4	83.4	108.3	101.8	0.72	0.85	0.86	0.75
Fallow 5	92.8	100.5	164.4	0.87	0.99	-0.61	-0.96
Fallow 6	99.1	98.5	85.1	0.98	0.97	-0.87	-0.81
Meadow 1	77	102	98.5	0.63	0.96	0.9	0.2
Meadow 2	48.8	158	98.4	0.32	0.27	0.95	0.94
Meadow 3	82.8	85.9	90.1	0.71	0.75	0.37	0.25
Meadow 4	82.2	112.9	101.7	0.7	0.77	0.86	0.75
Meadow 5	104.1	99.3	99.3	0.92	0.99	0.75	0
Meadow 6	87.8	106.1	98.4	0.78	0.88	0.83	0.62
Mean f ± SD	85.2 ± 11.7	107.5 ± 40.7	107.9 ± 30.1	0.76 ± 0.2	0.7 ± 0.4	0.11 ± 0.7	0.06 ± 0.8
Mean m ± SD	80.4 ± 18.1	110.7 ± 24.8	97.7 ± 4.0	0.68 ± 0.2	0.77 ± 0.3	0.78 ± 0.2	0.46 ± 0.4

activity through different size response observed in this study were in line with the previous suggestion that soil pH is the main determinant of soil microbial community structure (Fierer and Jackson, 2006; Wakelin et al., 2008; Lauber et al., 2009; Griffiths et al., 2011; Bahram et al., 2018). As Griffiths et al. (2008) have suggested, the soil biological stability "is governed by the physico-chemical structure of the soil through its effect on microbial community composition and microbial physiology for soil pH in this study. While it has recently been suggested that nutrients availability would increase resistance and resilience (Bardgett et al., 2017), we did not observe such an effect in our short-time respiration experiment. The relationship can be more complicated and case dependent. As observed by Liu et al. (2016), moderately N-fertilised soil had higher temperature sensitivity during winter when compared with control soils, however, the difference became non-significant during the vegetation period, which in part confirms our observation of higher change in respiration under cooling of richer nitrates fallow soil.

According to the literature, soil microbial K-strategist, oligotrophs, and food webs based on fungi shall be highly resistant, but not resilient. R-strategists, copiotrophs, and bacterial energy channel should, in contrast, be little resistant and highly resilient because "fast-growing" species (r-strategists) are more sensitive but recover more rapidly from a disturbance (Sigler and Zeyer, 2004; Orwin et al., 2006; Fierer et al., 2007; Bardgett et al., 2017). Higher resistance and adaptability to drought, but not resilience, were confirmed for grassland than for farmland due to presumed higher contribution of fungi into the food webs than of bacteria (de Vries et al., 2012). In line, we suspected that fallow communities resembled more copiotrophs than meadow communities because they responded quicker to resources which we observed previously (Chmolowska et al., 2017a, b), as well as to environmental modulators, as noted in the study. It is possible that in the case of pulse events, fallow soil microbial communities were prone to acclimation, while meadow soil microbial communities were more like stress-resistant sensu (Schimel et al., 2007).

The degree of respiration drop after drying obtained in this study was not high compared to the effect of temperature decrease. Resistance was close to the upper values and to 1.0, which means there was little impact of short-term drying on microbial activity. The methodology should be adapted further for increasing drying efficiency. Following the study by Orwin et al. (2006), overnight drying was used, while longer periods of drought were applied in other experiments, for example, 14 days in laboratory conditions (Chowdhury et al., 2011; de Vries et al., 2012). In the original work by Orwin et al. (2006), the calculated resistance and resilience of soil respiration varied from positive to negative values just within replicates of the same stage in a chronosequence, which corroborates the results obtained here. In addition to the methodological difficulties described above, different soils at the same moisture content may have different water potency (Boddy, 1986). The fickleness of the measured respiration under drying-rewetting exposed some of the characteristics of the resistance and resilience indices used. When the resistance gets below 0-1 and beyond 100-200% of control, using it can be problematic. A 50% and 150% change would give the same index value of 0.33; whereas five-fold increase of a measured response parameter (500% change of control) would yield a resistance of –0.6. Linear correlations might be questionable in a data set with a high variation of response indices, where positive and negative values of the index occur in a single data set. The authors of the index presented its operating range and explained some scenarios in which resilience is negative, e.g. in nutrients flush (Orwin and Wardle, 2004). Last but not least, because the index refers to absolute changes, hence, the absolute resistance index requires additional consideration as to whether smaller resistance means greater increase or greater decrease of a trait, which sometimes can be crucial from the biological point of view (e.g. stimulation, proliferation vs. exhaustion, reduction and degradation of a trait). The absolute index will hinder hormesis, e.g. in experiments using a gradient range of perturbation; when initial metabolic activation may occur depending on a dose, e.g. after pesticide treatment or watering.

In conclusion, the fallow soil respiration decreased relatively to 10ºC than meadow soil respiration, suggesting different temperature sensitivity of soil microbial communities in these two ecosystem types even though there were a number of similarities between them. This may cause differences in response to climate changes and the respiration rate during warmer winters in temperate grasslands, and potential for C sequestration although more evidence is needed for generalising the conclusion.

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The raw data from the temperature fluctuation experiment was deposited in a database together with all properties of the studied plots (Chmolowska, 2019).

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