









Tipping point in plant–fungal interactions under severe drought causes abrupt rise in peatland ecosystem respiration

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Abstract

Ecosystems are increasingly prone to climate extremes, such as drought, with long-lasting effects on both plant and soil communities and, subsequently, on carbon (C) cycling. However, recent studies underlined the strong variability in ecosystem's response to droughts, raising the issue of nonlinear responses in plant and soil communities. The conundrum is what causes ecosystems to shift in response to drought. Here, we investigated the response of plant and soil fungi to drought of different intensities using a water table gradient in peatlands—a major C sink ecosystem. Using moving window structural equation models, we show that substantial changes in ecosystem respiration, plant and soil fungal communities occurred when the water level fell below a tipping point of -24 cm. As a corollary, ecosystem respiration was the greatest when graminoids and saprotrophic fungi became prevalent as a response to the extreme drought. Graminoids indirectly influenced fungal functional composition and soil enzyme activities through their direct effect on dissolved organic matter quality, while saprotrophic fungi directly influenced soil enzyme activities. In turn, increasing enzyme activities promoted ecosystem respiration. We

show that functional transitions in ecosystem respiration critically depend on the degree of response of graminoids and saprotrophic fungi to drought. Our results represent a major advance in understanding the nonlinear nature of ecosystem properties to drought and pave the way towards a truly mechanistic understanding of the effects of drought on ecosystem processes.

KEYWORDS

biodiversity–ecosystem functioning, ecosystem shifts, fungal diversity, hydrolases, moving window structural equation model, oxidases, plant–soil feedbacks, threshold

1 | INTRODUCTION

Climate change is expected to increase the frequency and intensity of droughts (Berg et al., 2016; Dai, 2013). Such alterations in the environmental conditions can cause dramatic shifts in plant (Felton & Smith, 2017; Wellstein et al., 2017) and soil communities (Barnard, Osborne, & Firestone, 2013; Fuchslueger et al., 2016; Rousk, Smith, & Jones, 2013; de Vries et al., 2012) as well as on associated ecological processes (Fuchslueger, Bahn, Fritz, Hasibeder, & Richter, 2013; Hoover & Rogers, 2016; Kaisermann, Maron, Beaumelle, & Lata, 2015). These shifts can either gradually or suddenly alter ecosystem functions, often with important consequences for the goods and services that ecosystems can provide to humans (Bardgett, Freeman, & Ostle, 2008; Frank et al., 2015). However, the amount and rate of drought-induced ecological changes still remain highly uncertain (Hoover & Rogers, 2016). While the majority of studies report significant changes in the strength and/or direction of the relationship between biodiversity and ecosystem functions in response to drought (Fuchslueger et al., 2013; Rousk et al., 2013; Tilman, Isbell, & Cowles, 2014), others showed no shifts (Canarini, Carrillo, Mariotte, Ingram, & Dijkstra, 2016; Domínguez, Holthof, Smith, Koller, & Emmett, 2017; Hoover & Rogers, 2016; Sistla et al., 2014). These contrasting results suggest that climate–ecosystem relationships are often nonlinear and ecosystem functions sometimes remain close to a critical tipping point—a biophysical threshold where shifts in species diversity and interactions within communities may flip ecosystem functions to an alternative state, involving positive feedbacks among species at an unstable equilibrium (Briske, Fuhlendorf, & Smeins, 2006; van de Pol, Jenouvrier, Cornelissen, & Visser, 2017; Scheffer & Carpenter, 2003). The disturbance force ensuring that stress-induced changes in biodiversity effects on ecosystem functions remain close or cross a tipping point is, however, poorly understood (Baert, Janssen, Sabbe, & De Laender, 2016).

In ecosystems subjected to severe droughts, primary productivity typically decreases (Zhao & Running, 2010) and global soil carbon (C) loss increases (Crowther et al., 2016; Hicks Pries, Castanha, Porras, & Torn, 2017). Shifts in plant and soil biodiversity have emerged as the prominent determinants of such changes in ecosystem processes (van der Putten et al., 2013). However, there is less evidence of the functional identity of plants and/or soil microbiota (bacteria, fungi, archaea and protists) driving ecosystem shifts (Bardgett, Manning,

Morriën, & de Vries, 2013). Also, the evidence that drought affects ecosystem functions because of changes in the strength of interactions between specific plant and soil microbiota is mixed, at best (Fuchslueger et al., 2013; Loreau, 2001). As a result, it is still unclear how compositional changes in plant and soil communities can influence each other and to what extent these changes can drive ecosystems beyond an alternative state.

The response of a species to drought in one trophic level (e.g. plants) can cascade on species from other trophic levels (e.g. soil microbes). While in principle these shifts can lead to ecosystem changes, they do not necessarily have to, as they can have major biological impact without overhauling the essential properties and functions of the ecosystem (van de Pol et al., 2017). Compensatory mechanisms, such as the replacement of sensitive species by stress-tolerant species, may determine the magnitude of ecological changes in response to climate change (Lu et al., 2016). If a sensitive species is replaced by a stress-tolerant species that shares the same functional attributes, specific species-driven ecosystem functions should barely change. On the opposite, replacement of sensitive species by a stress-tolerant competitor with different functional attributes should disrupt existing species interactions, even create novel interactions, and hence alter ecosystem function (Fox, 2005).

Identifying explicitly the dynamic of plant–soil microbe interactions in response to drought and the amount of changes in plant–microbial feedbacks that pushes ecosystems towards an alternative state is essential for ecosystem management but still remains virtually unexplored. Here, we determine the level of drought that can modify plant–soil microbe interactions in a peatland ecosystem. Northern peatlands play an important role for the global C cycle as they represent a large but vulnerable pool of soil C (Dise, 2009). They store as peat over 500 Pg C mostly preserved by a high water table that limits decomposition (Yu et al., 2011). As such, peatlands are vulnerable to droughts and the interplay between plant and soil microbiota is central to how peatlands will respond to drought given their strong links with C cycle (Bragazza et al., 2016; Dieleman, Branfireun, McLaughlin, & Lindo, 2016; Robroek et al., 2016; Ward et al., 2015). Any shifts in the direction and/or strength of plant–soil microbe interactions could destabilize the peatland C balance (Kuiper, Mooij, Bragazza, & Robroek, 2014). Likely, a prethreshold state within soil and/or plant components must exist and necessarily reached before peatland functions shift in response to drought.

In this study, we use data from a unique water level manipulation experiment in a Polish peatland where different drought intensities were simulated. We determined the diversity and functional community composition of plants and fungi, soil enzyme multifunctionality, dissolved organic matter quantity and quality, and ecosystem respiration. We focused on fungal diversity and community composition in the light of the role that fungi play as both decomposers of organic matter and root-associated mediators of below-ground C transport and respiration (Clemmensen et al., 2013). We specifically tested which level of compositional and functional changes in plant and fungal communities in response to drought will lead to a shift in ecosystem respiration, and whether there are causal interactions between tipping events among trophic groups. We hypothesized that (1) drought will initiate a domino-like event within plant and fungal communities, including their interactions, that will shift peatland function. Consequently, we expect (2) that the response of fungi to drought will trigger the first shifts in soil enzyme activity that will be exacerbated by the simultaneous response of plant community. In turn, (3) higher soil enzyme activity will stimulate ecosystem respiration by a domino effect. Our study emphasizes the importance of considering domino-effects within plant–soil interactions to better understand and predict how a changing climate can shift ecosystem function.

2 | MATERIALS AND METHODS

2.1 | Study site and water table manipulation

We conducted the experiment in Linje mire (northern Poland, 53°11'15"N, 18°18'34"E, ca. 6 ha), a poor fen with ombrotrophic vegetation (Lamentowicz et al., 2016). The moss layer was dominated by *Sphagnum fallax* while the herbaceous layer was characterized by *Betula nana* the graminoids *Eriophorum vaginatum*, *Carex rostrata* and the ericoid *Oxycoccus palustris*. The water table manipulation consisted of three water table treatments (Wet, Ambient and Dry) randomly assigned to 18 homogeneous plots (1 m²) in terms of vegetation (i.e. six plots per combination) (Lamentowicz et al., 2016). The 18 plots were spread over a hectare with minimal distance between two plots of c. 1 m and maximal distance of c. 25 m. The water level manipulation was obtained by either lowering (Wet) or raising (Dry) the top 30 cm of the vegetation carpet. In the Wet plots, we first cut and removed four peat monoliths (50 × 50 × 30 cm; l × w × h). Then, we dug up 10 cm of the underlying peat and kept it aside. The peat monoliths were finally placed back on the same position so that the moss surface was now 10 cm lower in comparison to the Ambient plots. In the Dry plots, a similar monolith removal was performed. In these plots, however, the 10 cm peat material previously removed from the Wet plots was added underneath the vegetated monolith before they were placed back. This procedure raised the moss surface by 10 cm in comparison to the Ambient plots. In Ambient plots, the four peat monoliths were cut, removed and replaced on the same position. All 18 experimental plots were bordered by a plastic sheeting (15 cm height) to

maintain the moss carpet. An elevated boardwalk was constructed to prevent damages from trampling during the measurements and the maintenance works. We further selected six other plots that remained untouched to test for the peat cutting and moving effects on the biotic and abiotic variables that were measured. Overall, we did not find significant effects of the cutting and moving phases for most of the measured variables in this study (Table S1). The water table manipulation experiment started in mid-May 2012 but we left the system recovering for 1 year before starting the measurements. We collected samples after 12 (mid-May 2013), 15 (mid-August 2013), 18 (early November 2013) and 27 (mid-August 2014) months from the beginning of the experiment. These dates were chosen to both coincide with natural water level variations (Figure 2) and different seasons.

2.2 | Local-scale environmental conditions

In each plot, air (30 cm above the *Sphagnum* carpet; HOBO U23 Pro v2 data loggers) and peat (5 cm depth; 5TM sensor Decagon Devices, Inc., USA) temperatures were recorded continuously every 10 min (Lamentowicz et al., 2016). Water Table Depth (WTD) was recorded using HOBO U20-001-01 pressure data loggers (Onset Computer Corporation, USA) every 3 hr in four piezometers within the experimental site. WTD for each plot was then corrected using the averaged WTD across the experimental site and the averaged distance from the *Sphagnum* layer to the water table depth of each plot (corrections were adjusted several times per year). As a result, strong negative WTD values indicate dry conditions, while WTD values close to 0 indicate wet conditions. Precipitation was recorded continuously throughout the entire study period using a tipping bucket Rain Gauge Smart Sensor (S-RGB-M002, Onset Computer Corporation, USA) with a resolution 0.2 mm. Vapour Pressure Deficit (VPD) was calculated by following Abteu and Melesse (2013).

2.3 | Vegetation survey

We assessed the seasonal dynamic of plant species cover at each date in each plot using pictures taken in 50 × 50 cm subplots (Buttler et al., 2015). We took high-resolution images based on four 25 × 25 cm quadrats and used a grid of 100 points laid on each quadrat to quantify species overlaying the grid intersects (Buttler et al., 2015). This technique did not allow accounting for the vertical biomass distribution and may underestimate the abundance of certain species such as *Sphagnum fallax*. However, the bias was similar in each plot, thus making species frequencies comparable among treatments, and did not further influence the response of plant species to treatments. We especially distinguished the graminoid cover from the ericoid cover as these two plant types differ in productivity, litter, root inputs and plant–fungal associations, and as such may have different effects on ecosystem C dynamic (Robroek et al., 2015, 2016; Walker, Ward, Ostle, & Bardgett, 2015; Walker et al., 2016).

2.4 | Water-extractable organic matter quantity and quality

Water-extractable organic matter (WEOM) was extracted from *S. falax* shoots (100 g; 0–8 cm depth) collected at each sampling period in all manipulated plots following Delarue et al. (2011). The water extract was then prefiltered through a gauze membrane (1 mm pore size) to remove *Sphagnum* remains and filtered at 0.45 μm through a glass fibre filter. *Sphagnum* remains were dried at 60°C for 48 hr. Dissolved organic carbon and nitrogen (WEOC and WEON respectively) concentration in the WEOM were quantified by combustion using a TOC analyser (Shimadzu TOC-V CPH) and expressed as mg C or N g^{-1} dw of *Sphagnum*. WEOM aromatic content and molecular weight was measured using specific UV absorbance at 254 nm (SUVA_{254}) and absorbance measurements between 250 and 465 nm (15 wavelengths in total) in 200 μl sample aliquots in 96-well quartz microplate using a BioTek SynergyMX spectrofluorometer (Jaffrain, Gérard, Meyer, & Ranger, 2007). Spectral slopes ($S_{250-465}$, nm^{-1}) were calculated using linear least squares regressions with Ln-transformed absorptions. High SUVA_{254} value indicates a high aromatic content while high $S_{250-465}$ values indicate low molecular weight material and/or decreasing aromaticity of the WEOM (Hansen et al., 2016).

2.5 | Fungal DNA analyses and bioinformatics

DNA was extracted with MoBio Power Soil DNA isolation kit (Carlsbad, CA USA) according to the instructions provided by the supplier. PCR amplification follows the protocol of Amaral-Zettler, McCliment, Ducklow, and Huse (2009). The eukaryotic PCR primers 1380F (CCCTGCCHTTTGTACACAC) and 1510R (CCTTCYGCAGGTTCCACTAC) targeting V9 region of the SSU rRNA gene were used. Forward primers were tagged on the 5' end with a 10 nucleotides strand, specific to each sample. The PCR reactions mix (20 μl reaction volume) consisted of 1 U GoTaq[®] G2 DNA Polymerase (Cat No.: M7845, Promega, Switzerland), 1X Colorless GoTaq[®] Reaction Buffer, 300 μM dNTPs (Cat No.: U1420, Promega, Switzerland) and 0.3 μM of each primer. The PCR conditions were as follows: initial activation step at 95°C for 3 min, followed by 30 three-step cycles (consisting of 94°C for 30 s, 57°C for 30 s and 72°C for 1 min) and a final 10 min extension at 72°C. The PCR products were purified with Wizard[®] SV Gel and PCR Clean-Up System (Promega, Madison, WI USA). Sequencing was performed with Illumina's HiSeq technology, using V3 chemistry by Fasteris SA, Switzerland.

The bioinformatics pipeline included quality check (Phred score filtering, elimination of reads without perfect forward and reverse primers) and removal of rare sequences (sequences occurring less than three times in the full data set or in less than two distinct samples). Sequences were clustered into Operational Taxonomic Units (OTUs) with the Swarm algorithm (Mahé, Rognes, Quince, de Vargas, & Dunthorn, 2014), and the number of reads of a given OTU counted in each sample. The most abundant sequence of each OTU was selected as the representative to be aligned (BLASTn) against the UNITE (www.unite.ut.ee) data base (Kõljalg et al., 2013). For each query, we thus considered the

10 best-matching references to annotate our global sequences as accurately as possible (Tedersoo et al., 2014). OTUs with e -values $< e^{-50}$ and bit-scores > 100 were assigned to species level if homology was 100%–97%, genus for 96.9%–93%, family or order for 92.9%–80%. OTUs with e -values $> e^{-20}$ were considered as “unknown”, while those with e -values between e^{-20} and e^{-50} were carefully checked against the 10 best matches and implemented in the PlutoF workbench (Kessy Abarenkov et al., 2010) for accurate assignment. Identified OTUs were further assigned to functional guilds using FUNGuild (Nguyen, Song, Bates, Branco, & Tedersoo, 2016) with some modifications according to literature: undefined root endophyte, ectomycorrhizal fungi, ericoid mycorrhizal fungi, lichenized fungi, plant pathogen, animal pathogen, mycoparasite, undefined saprotroph, leaf/litter saprotroph, wood saprotroph and unknown. All details are provided in Table S2.

2.6 | Soil enzyme multifunctionality and ecosystem respiration

The soil enzyme multifunctionality was analysed using a catabolic response profile approach by measuring enzymatic activities of three hydrolytic and two oxidative C-degrading enzymes, one hydrolytic C/N-degrading enzyme, three aminopeptidases (N-cycle) and one acid-phosphatase (P-cycle; see Table S3 for details). Enzyme activities were quantified in *Sphagnum* litter (0–8 cm depth; approximately 300 g of fresh *Sphagnum* litter per plot) as relative enzyme activity, that is, enzymes activities assessed under saturating substrate conditions and stable temperature (25°C). Enzymes were quantified in microplates following Jassey, Chiapusio, Gilbert, Toussaint, & Binet (2012) and Jassey et al. (2016). Fluorescence of hydrolases was monitored spectrophotometrically with an excitation wavelength of 365 nm and emission detection at 450 nm (BioTek, SynergyMX) while oxidation rates of oxidases were monitored spectrophotometrically at 600 nm (Table S3). All enzymatic activities were converted to nanomoles per gram dry weight per min ($\text{nmol min}^{-1} \text{g}^{-1} \text{DM}$).

Ecosystem CO_2 respiration (R_{ECO}) was measured at the same periods using a dynamic closed chamber system consisting of an infrared gas analyser EGM-4 and a darkened net canopy assimilation chamber CPY-4 (PP-Systems International, Inc., USA). Each measurement was carried out by putting the chamber on a 20 cm diameter collar inserted into a *Sphagnum*-dominated (vascular plant free) surface at the beginning of the experiment. The CO_2 concentration was recorded over 2 min at 4 s intervals. At each field campaign, measurements were performed repeatedly during a full day, using three analysers and a stochastic sampling scheme for the path of plot measurements. Fluxes are expressed in $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$.

2.7 | Numerical analyses

2.7.1 | Deconstruction of trend and seasonal figures in climatic time series

Given WTD variations were strongly collinear with sampling dates (Figure 2), we deconstructed the WTD time series into its trend and

seasonal components. The trend component reflects the long-term progression of the series such as an increasing or decreasing direction in the data. The seasonal component reflects the seasonal patterns in the time series (e.g. repeated periodic fluctuations). The seasonal component was calculated through a fixed period of 92 days. We extracted these two components using additive models (Brockwell & Davis, 2016). The trend component was removed by smoothing the time series using a centred moving window average (period of 92 days). Removing the trend from the time series resulted into a new time series where seasonal figure is clearly exposed. From this resulting seasonal figure, we computed an average seasonality for each time unit (day), over all periods. The seasonal figure was finally centred. We repeated that procedure on each plot and each climatic time series (WTD, temperature and VPD) and averaged the seasonal figure of each time series as a proxy for “climatic seasonality.” Deconstruction of the time series was achieved using the `DECOMPOSE` R package (R Core Team 2015).

2.7.2 | Response of belowground components to drought

We evaluated the effect of water level treatments, trend in WTD and climatic seasonality on plants cover, enzymatic

activities, fungal richness and WEOM properties (WEOC, WEON, $SUVA_{254}$, $S_{250-465}$) over time (after 12, 15, 18, 27 months) using linear mixed-effect models. All models were fitted including plot nested into climatic seasonality as a random effect on the intercept to correct for the inflation of the residual degrees of freedom that would have occurred if we were using repeated measurements as true replicates (Pinheiro & Bates, 2000). The `nlme` R package was used to run these models. The Tukey's HSD test was used for post hoc comparisons among treatments. We then performed nonmetric multidimensional scaling (NMDS) and a principal component analysis (PCA) for a specific interpretation of fungal OTU communities' structure and enzyme activities' patterns (both data sets were log-transformed), respectively, at each period and for each treatment. Sites scores from the PCA were further used as a proxy for enzyme multifunctionality (M_{enz})—an averaging method that attempts to summarize multiple enzyme activities so that high values of M_{enz} mean high values of many of, but not necessarily all, the enzyme activities included (Maestre et al., 2012). We extracted the site scores from these two ordinations and subjected them to linear mixed-effect models, as described above, to interpret significant interactions between shifts in fungal communities (or enzymatic patterns) and WTD variations.

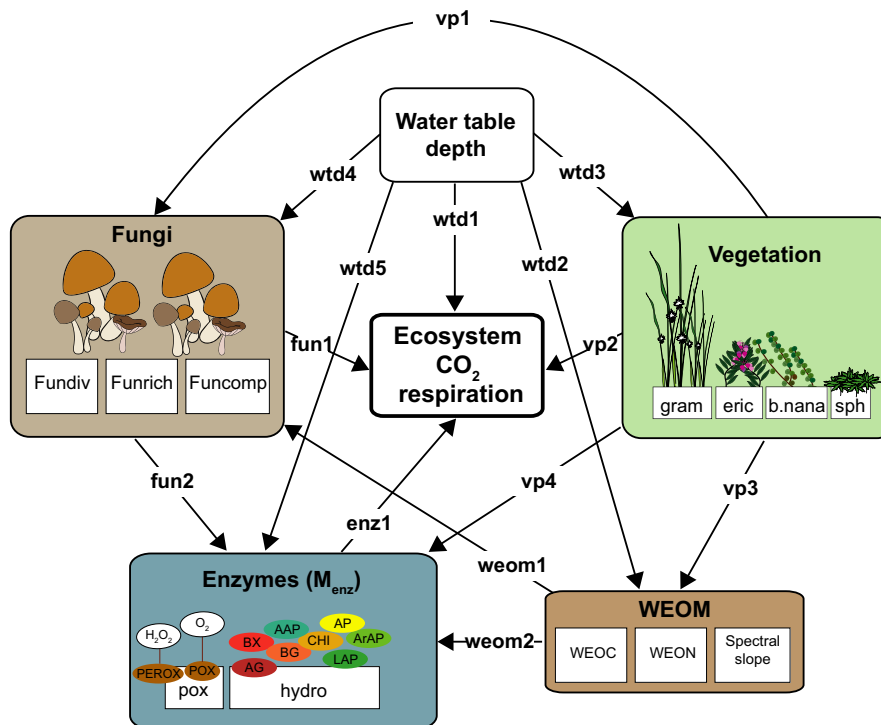


FIGURE 1 A priori conceptual structural equation model (SEM) depicting pathways by which water table depth, dissolved organic matter (WEOM), vegetation, fungi and enzymes may influence ecosystem CO_2 respiration (see Table 1 for detailed hypotheses). For simplicity, we only show global pathways by variable category (e.g. fungi); a detailed version of the model is given in Fig. S1. Letters correspond to hypothetical pathways (Table 1). Fundiv = fungal diversity; Funrich = fungal richness; Funcomp = fungal community composition (NMDS axis 1); gram = graminoid cover; eric = ericoid cover; b.nana = *Betula nana* cover; sph = *Sphagnum* cover; weoc = water-extractable organic carbon; weon = water-extractable organic nitrogen; Spectral slope = spectral slope of the WEOM; pox = phenoloxidases (POX + PEROX); hydro = hydrolases (PCA axis 1 computed on BX, AG, BG, CHI, LAP, ArAP, AAP, AP). Hydrolases abbreviations can be found in Table S4 [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

2.7.3 | Identifying tipping points

We used broken-line regression models (segmented method) to identify possible tipping points in key descriptors such as enzyme multifunctionality, plant cover and $S_{250-465}$. This method is the most adequate method to determine tipping points in ecosystem components (Vanacker, Wezel, Payet, & Robin, 2015). Segmented method tests whether the relationship between two variables is piecewise linear, namely represented by two or more straight lines connected at unknown changing points. The output of this analysis provides tipping point estimates with relevant uncertainty; in other words, it gives estimated, and not fixed, changing points with a range where a tipping point could be situated. We used `SEGMENTED` R package (Muggeo, 2008) to perform these analyses. We further parsed fungal OTU community response to water level gradient using Threshold Indicator Taxa Analysis (TITAN). This method can reveal sharp, non-linear transitions in community data (see Baker & King, 2010 for a full description). Briefly, TITAN identifies the optimum changing point of every species frequency and abundance along a continuous variable x using the indicator species analysis approach that partition the data along x . Indicator species analysis measures how well a taxon is associated with the group either above or below a certain value on an environmental gradient (the water level in this case) (Dufrêne & Legendre, 1997). Each taxon is assigned to an indicator value (IndVal scores) that take into account the frequency of occurrence, the proportional abundance and directionality of taxa responses in these two groups. IndVal scores are scaled between 0% and 100% with a value of 100 indicating that a taxon was collected in every sample within a group and not the other group. In TITAN, IndVal scores are calculated for all species for all possible change points along the environmental gradient (every observed value of x are candidate change points), with permutation tests to assess the uncertainty in these scores (Baker & King, 2010). TITAN empirically distinguishes increasing (positive response) and decreasing (negative response) taxa at each candidate change points, whereas taxon-specific response magnitude is normalised as z scores (based on SD and mean of permutations) and sums for positive [$\text{sum}(z+)$] and negative [$\text{sum}(z-)$] responses for each possible change point along the gradient. TITAN thus detects changes in species distributions along a gradient and assesses synchrony among species change points as evidence for community thresholds. $\text{Sum}(z)$ peaks highlight values of the environmental variable around which many taxa exhibit strong directional changes in abundance representing community thresholds. The quality and estimation of the critical tipping point area in fungal community was tested using 1,000 permutations and 500 bootstrap replicates (resampling with replacement) using custom `TITAN2` R package (Baker & King, 2010). We transformed the raw OTU sequence data set using a centred log transformation.

2.7.4 | Drivers of ecosystem CO_2 respiration

The response of ecosystem respiration to drought can be determined by numerous interconnected biotic mechanisms that operate

simultaneously. One approach to studying such complex interactions is structural equation modelling (SEM; Grace, Adler, Harpole, Borer, & Seabloom, 2014). Following current knowledge of peatland responses to drought, we developed an a priori model of hypothesized relationships within a path diagram (Figure 1, Table 1), allowing a causal interpretation of the linkages between WTD, plant and fungal community composition, enzyme multifunctionality, WEOM quality and R_{ECO} (Grace & Keeley, 2006). We first ran the a priori model on the entire data set to identify the main drivers of R_{ECO} . Our SEM approach was semi-exploratory in that while we worked to address general hypotheses embodied in a path diagram, the precise variables (e.g. Graminoid cover vs. *Sphagnum* cover) to use were determined empirically based on the outputs of the full model (Fig. S1) and by step-wise exclusion/selection of variables, as estimated by Akaike Information Criterion (AIC) (Grace et al., 2016). The critical tipping point area among the plant–soil microbial drivers of R_{ECO} was identified using a smoothing SEM approach. Smoothing was achieved by running moving window SEMs along the WTD continuum. First we ranked the entire data set from lower to higher WTD. Then, we sequentially changed the subset of plots (=window) by adding the next more wet plot and discarding the least wet one. We used a window of 26 consecutive samples across the WTD gradient (total of transits = 29) and quantified for each window the variation in R_{ECO} (R -square value) explained by the model. Multiple window sizes were tested initially, all showing similar patterns; the 26 sample window size was, however, optimal for interpretation without losing too much fit in SEMs. Path analyses were performed using the `SEM` R package and moving window SEMs using personal R codes (`MOVINGSEM` function available at <https://doi.org/10.5281/zenodo.913424>).

The degree of response of ecosystem respiration's drivers (D) to drier conditions was calculated using standardized effect sizes (SES), as follow:

$$\text{SES}_D = \frac{D_i - D_{\text{wet}}}{\text{SD}(D)}$$

where D_i is a value of D along the water level gradient, D_{wet} the value of D that corresponds to the wettest WTD along the gradient and $\text{SD}(D)$ the standard deviation of D . SES was calculated for each driver of ecosystem CO_2 respiration found in SEM and then averaged.

3 | RESULTS

3.1 | Seasonal variation of hydrological and climatic parameters

The WTD significantly differed between the three water level treatments ($p < .001$; Figure 2a). On average, the water level was 37% lower in the Dry treatment than in the Wet treatment ($p < .001$) and 28% lower than in the Ambient treatment ($p < .001$). Similarly, the water level in Ambient treatment was 13% lower than in the Wet treatment ($p < .01$). Furthermore, the water level significantly and similarly decreased over time in all treatments (date: $p = .0001$;

TABLE 1 Components of hypotheses represented by the structural equation model

Path	Causal hypothesis
wtd1–5	Decreasing water level directly promotes ecosystem respiration (wtd1). Drop in water table directly shifts WEOM quality (wtd2) and/or promotes enzyme activities due to higher oxygen availability (wtd3; Fenner & Freeman, 2011). Lower water level influences the fungal community composition through effects in fungal diversity and fungal richness (wtd4; Jaatinen et al., 2007). Vascular plants (graminoids and ericoids) respond positively to decreasing water level while <i>Sphagnum</i> responds negatively (wtd5)
vp1	Changes in vegetation community composition influences fungal community composition. For instance, an increase in ericoids may favour ericoid mycorrhizal fungi
vp2	Vegetation directly influences ecosystem CO ₂ respiration through autotrophic respiration
vp3	An increase in vascular plants promotes microbial enzyme activities, likely through root exudates (Dieleman et al., 2017)
vp4	Shifts in plant community composition influence the quantity and/or quality of the water-extractable organic matter (WEOM) through the release of organic compounds (Robroek et al., 2016)
fun1	Fungi directly influences ecosystem CO ₂ respiration through heterotrophic respiration
fun2	Fungal community composition influences microbial enzyme activities. For example, an increase in saprotrophic fungi promotes enzyme activities
weom1	Shifts in the quantity and/or quality of WEOM influence fungal community composition
weom2	Shifts in the quantity and/or quality of WEOM influence enzyme activities. For instance, an increase in simple organic compounds promotes hydrolase activities while an increase in recalcitrant compounds such as polyphenols increases oxidases (Robroek et al., 2016)
enz1	Increasing microbial enzymatic activities promote ecosystem CO ₂ respiration; the more active microbes are, the more they respire (Fenner & Freeman, 2011)

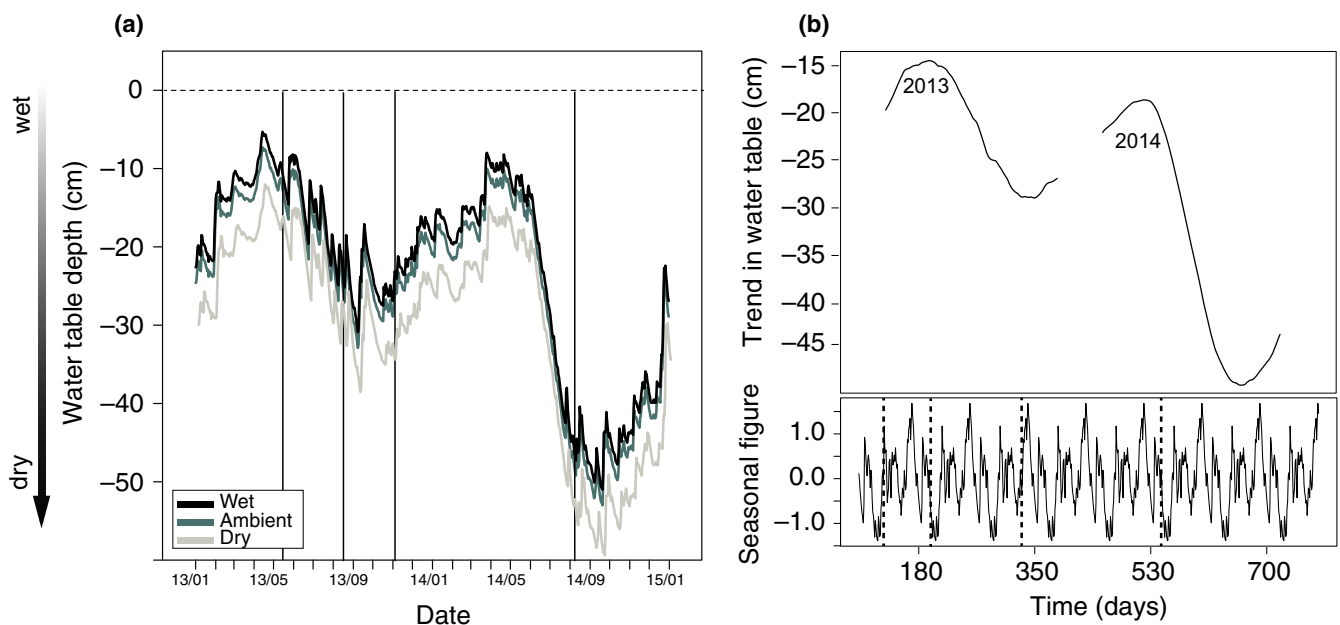


FIGURE 2 Response of the water table depth (WTD) to manipulation in 2013 and 2014. (a) Effect of water level manipulation over time on water level depth. Vertical lines represent the sampling dates. (b) Decomposition of the water level depth time series into its trend and seasonal figure (see Section 2). Trends are different in 2013 and 2014 while the seasonal figure remain similar between 2013 and 2014. Dashed lines represent the different sampling dates [Colour figure can be viewed at wileyonlinelibrary.com]

date \times WTD treatment: $p = .66$). Especially, we observed an equally important drop of the water level in all treatments during summer 2014, with a mean drop of 21 cm between mid-May 2014 and mid-August 2014 (Figure 2a,b). Greater vapour pressure deficit between 2013 and 2014 further corroborates these results by indicating warmer and drier conditions in 2014 than in 2013 (Fig. S2). Overall, the temporal and natural fluctuations of water table depth spanned over a gradient from -40 to -5 cm. Decomposition of the trend and seasonal components of WTD showed that temporal variations of WTD

remained similar in 2013 and 2014 but with a decreasing trend in 2014 compared to 2013 (Figure 2b).

3.2 | Response of plant community to water table depth

Trend in water table depth had larger effects on plant cover than climatic seasonality (Table S4). Under Ambient conditions, vascular plants benefited from decreasing water level with an increase in

their cover by 47% after 27 months compared to the beginning of the experiment ($p < .01$, Figure 3a,b). This increase was particularly driven by the graminoids ($F_{1,70} = 68.8$, $r = .70$ and $p < .01$), especially *Eriophorum vaginatum* (Fig. S3). On the opposite, decreasing water level reduced *Sphagnum* cover in Ambient plots by 77% after 27 months from the beginning of the experiment ($p < .01$). These changes decreased the moss/graminoid ratio from 8 to 0.17 from the beginning to the end of the experiment. Beyond natural variations of water table, vascular plants and *Sphagnum* either benefited or suffered from water level manipulations. *Sphagnum* plants always had a lower cover in Dry treatments compared to Ambient and Wet ($F_{2,64} = 45.1$, $p < .001$), especially after 27 months when they almost disappeared from the plant community (Figure 3a). On the opposite, wetter conditions promoted *Sphagnum* cover that was on average 33% higher in Wet plots compared to Ambient plots ($p < .01$). We found opposite effects for vascular plants as their cover was on average 10% higher in Dry treatment and 18% lower in Wet treatment compared to Ambient conditions respectively ($F_{2,64} = 24.6$, $p < .001$; Figure 3a). Particularly, we found that the dominant ericoid *Oxycoccus palustris* had a consistent lower cover in Wet plots compared to Ambient and Dry plots (Fig. S3). We further found that the vascular plant community shifted from an ericoid dominance at the beginning of the experiment towards an equal dominance between graminoids and ericoids (Fig. S3a,b) along with decreasing water table, particularly at a water level of c. -20 cm (Figure 3b).

3.3 | Response of dissolved organic matter (WEOM) to water table depth

The quantity and quality of WEOM significantly varied during the season but they were mainly influenced by trends in WTD (Table S4). Under Ambient conditions, both WEOC ($r = .46$, $p < .01$) and the spectral slope ($S_{250-465}$) of WEOM ($r = .71$, $p < .01$, Figures 4a and S3c) increased along with the decrease in the water level, while WEON remained stable ($p = .66$; Fig. S3d). $SUVA_{254}$ decreased along with decreasing water level ($r = .56$, $p < .01$; Fig. S3e). While WEOC, WEON and $SUVA_{254}$ did not respond to water level treatments, the spectral slope ($S_{250-465}$) of WEOM was lower in Wet plots compared to Ambient and Dry conditions, especially after 15 and 18 months (Figure 4a). Altogether, these results indicate a lower aromaticity and/or lower molecular weight of WEOM along with decreasing WTD, and that Dry treatment enhanced such trend. Specifically, WEOM was less and less aromatic until a water level of c. -24 cm was reached and then it remained more stable (Figure 4b). This changing point was concomitant with the point where graminoids and ericoids started to be codominant (Figure 3b).

3.4 | Response of fungal soil community and enzyme multifunctionality to water table depth

Fungal OTU richness under Ambient treatment increased with the natural decreasing of WTD ($r = -.76$, $p < .01$). The fungal OTU

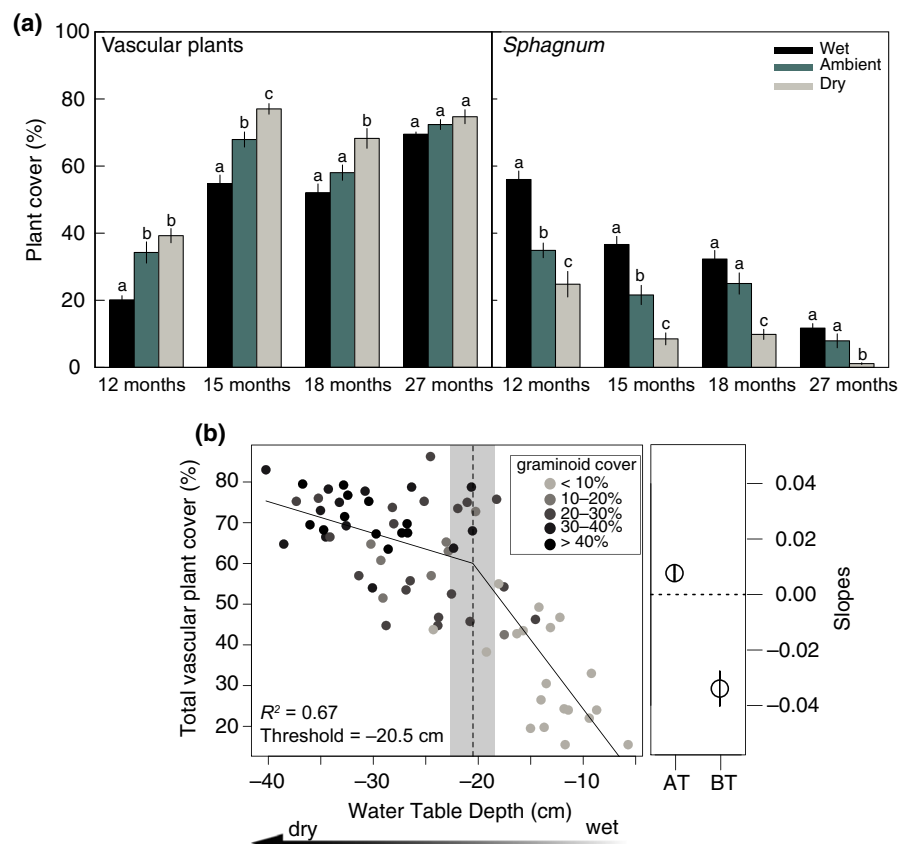


FIGURE 3 Response of vascular plants and *Sphagnum* mosses to water level manipulations and water level gradient. (a) Effect of water level manipulation on total vascular plant and *Sphagnum* covers at each sampling date (detailed statistics can be found in Table S4). Letters indicate significant differences among water level treatments at a specific period. (b) Response of total vascular plant cover to water level gradient. Slope coefficients from each side of the tipping point are shown on the left. AT = Above threshold; BT = Below threshold. Dots colours indicate the total graminoid cover. Confidence interval of the threshold is shown in grey [Colour figure can be viewed at wileyonlinelibrary.com]

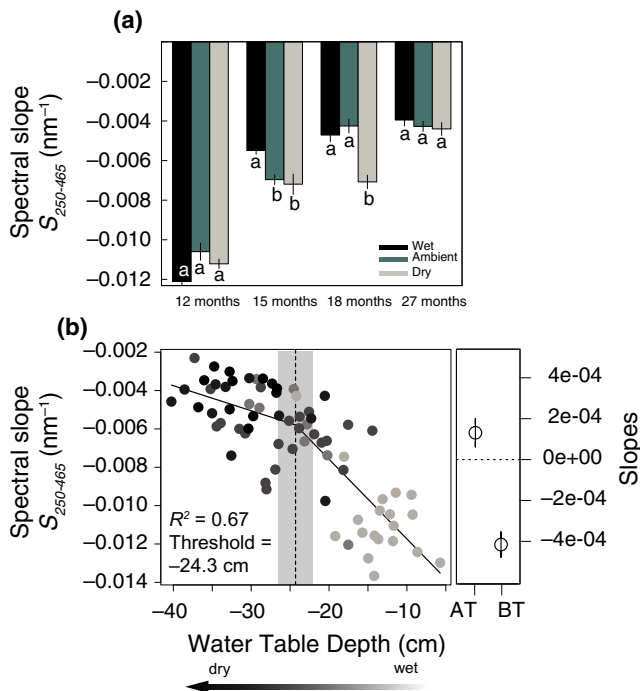


FIGURE 4 Response of dissolved organic matter quality to water level manipulations and water level gradient. (a) Effect of water level manipulation on water-extractable organic matter (WEOM) quality (spectral slope $S_{250-465}$) at each sampling period (detailed statistics can be found in Table S4). Letters indicate significant differences among water level treatments at a specific period. (b) Response of WEOM quality (spectral slope coefficient, $S_{250-465}$) to water level gradient. Slope coefficients from each side of the tipping point are shown on the left. AT = Above threshold; BT = Below threshold. Dots colours indicate the total graminoid cover as in Figure 3. Confidence interval of the threshold is shown in grey [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

richness was also consistently higher in Dry treatment compared to Ambient and Wet treatments respectively ($F_{2,49} = 9.8$, $p = .03$; Fig. S4). NMDS analysis showed that fungal OTU composition differed over the natural WTD gradient (NMDS axis 1) and among Wet, Ambient and Dry treatments (NMDS axis 2; Fig. S5a). Using threshold indicator taxa analysis (TITAN), we identified 205 OTUs (out of 489) that showed a significant changing point along the WTD gradient (Figure 5a), predominantly at WTD between -28 and -21 cm. Among them, 174 OTUs were closely related to dry conditions (Figure 5a), especially saprotrophs (Figure 5b). As a result, fungal community composition strongly converged at a WTD of c. -26.8 cm (Figure 5c) indicating a community-level ecological threshold.

Trend in water table depth had larger effects on enzymatic activities than seasonality (Table S4). PCA analysis showed that enzymatic patterns differed over the natural WTD gradient (PCA axis 1) and among Wet, Ambient and Dry treatments (PCA axis 2; Fig. S5b). In particular, Dry treatment tended to promote hydrolytic C and chitinase enzyme activities compared to Wet treatment, while peptidases and phosphatase either decreased (e.g. LAP, -16%) or remained stable (Fig. S6, Table S4). Segmented model analysis

further showed that enzyme multifunctionality strongly increased once the water level crossed the depth of -25.8 cm (Figure 5d). Such a pattern was mostly driven by the response of hydrolytic enzymes (Fig. S7), which all showed a significant change point between -25 and -28 cm along the WTD gradient (Table S5). Oxidases either decreased (peroxidases, $r = -.61$, $p < .001$) or remained stable (phenoloxidases, $r = .004$, $p = .94$) along the water level gradient.

3.5 | Tipping point in ecosystem CO₂ respiration

Ecosystem CO₂ respiration was more influenced by the trend in water table depth than climatic seasonality (Table S4). Particularly, ecosystem respiration suddenly increased once the water level dropped below c. -25 cm (Figure 5e), but no effects of water level treatment were found (Fig. S8). Moving window SEMs showed that the response of ecosystem CO₂ respiration (R -squared value) to shifts in plant and fungal communities, including their interactions with enzymes, could be divided into three phases: a first phase with low R^2 values (phase 1, WTD = -16 to -21 cm), a second transition phase with increasing R^2 values (phase 2, WTD = -21 to -24 cm) and a third phase with high R^2 values (phase 3, WTD > -24 cm; Figure 6a, Table S6). These three phases correspond to different water table levels ranged from wet to dry respectively. When averaged for each phase, SEMs showed that increased ecosystem CO₂ respiration was mostly mediated by the direct influence of graminoids on WEOM quality, which in turn, influenced fungal community composition and enzyme activities (Figure 6b). SEMs further showed that the influence of fungal community composition on ecosystem CO₂ respiration was either direct, or indirect through enzyme multifunctionality. In general, SEM moving window analysis showed progressive bifurcations from the “wet” towards the “dry” phase in the drivers of ecosystem CO₂ respiration. First, the WTD had an effect on plant and fungal communities (Figure 6b, Table S6). Second, graminoids influenced WEOM quality, while the effect of WEOM quality on enzyme multifunctionality increased over the gradient. Finally, the effect of fungi and enzymes on ecosystem CO₂ respiration increased in intensity along the water level continuum (Figure 6b, Table S6). More precisely, we found that although the drivers of ecosystem respiration responded to lowering water level, their effect on respiration was significant only once a threshold was passed in their degree of response > 0.3 (Figure 6c).

4 | DISCUSSION

The likelihood of sharp functional shifts in natural ecosystems is expected to increase as climate change intensifies (Hoover & Rogers, 2016), in particular, in response to repeated drought events (Angeli, Ferrell, & Sontag, 2004). Pinpointing the specific triggers of such ecosystem shifts is challenging. One of the big question is which combination of climatic and biotic changes causes ecosystems to cross a tipping point in their functioning. In this study, we identified

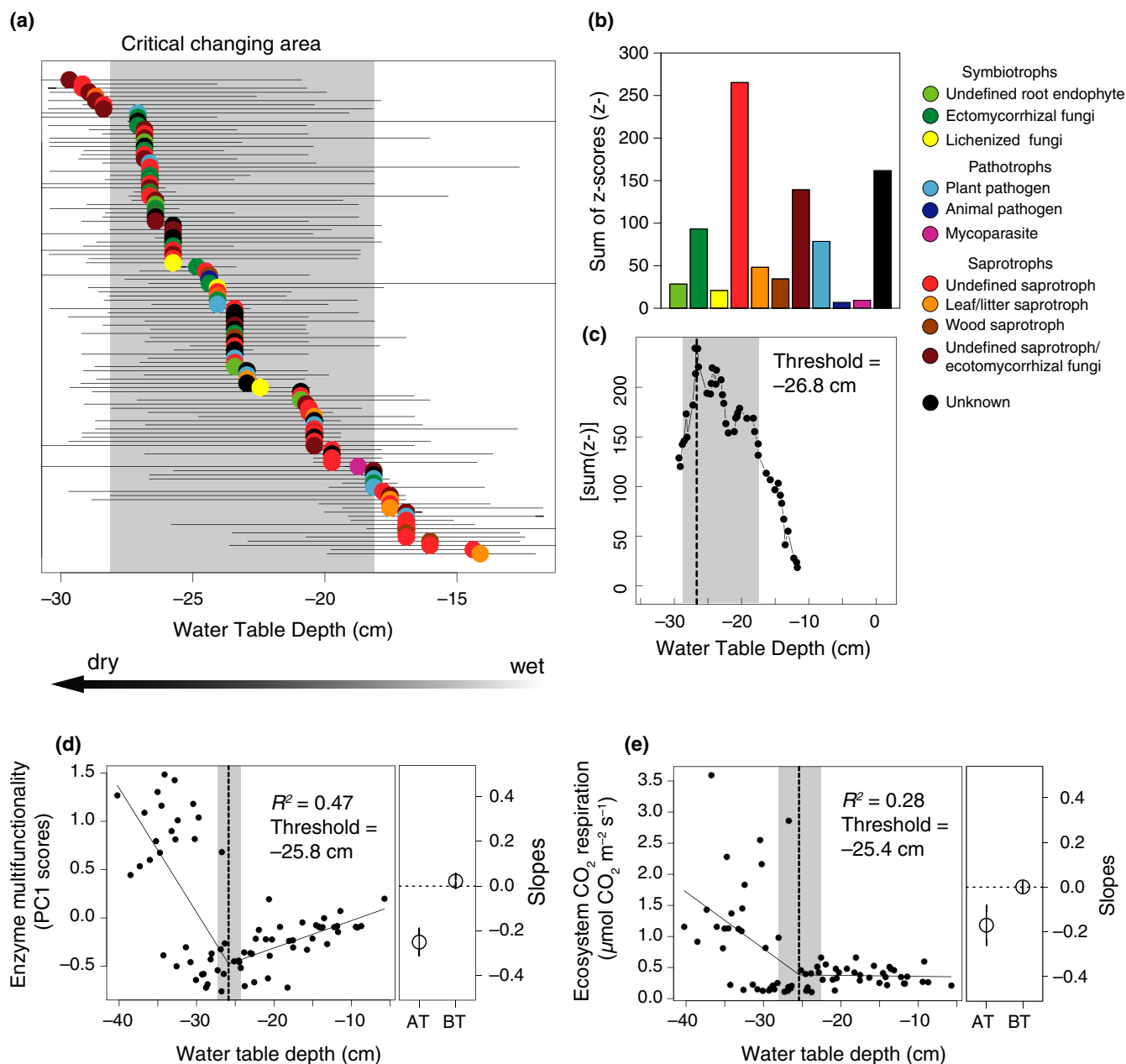


FIGURE 5 Response of belowground components to water level gradient. (a) Fungal species change points along the water level gradient (purity >99%, $p < .05$ in >99% bootstraps) showing 5% and 95% bootstrap percentiles; dot colours reflect the function of the species. Only species that reduced in abundance with wet conditions are shown. Critical changing area reflects the 5%–95% bootstrap percentile range (in grey) of community change point (see subset c). All details about OTUs are given in Table S2. (b) Sum of z scores (z-) over all the gradient according to fungal functions. (c) Community change point for OTUs reduced in abundance along with wet conditions showing community threshold (dotted line) at max([sum(z-)]) and 5%–95% bootstrap percentile range. [sum(z-)] values represent the sum of responses for each possible change point along the gradient. (d–e) Enzyme multifunctionality and ecosystem CO₂ respiration as a function of water level depth. Slope coefficients from each side of the tipping point are shown on the right. AT = Above threshold; BT = Below threshold. Confidence intervals of the thresholds are shown in grey

how the individual response of plants and soil fungi along a moisture gradient pushes peatland ecosystem respiration towards an alternative state. The results confirmed our hypothesis that drought can lead to a domino-like event where positive feedbacks among plants and fungi force the system towards a critical tipping point amplifying small initial changes in ecosystem CO₂ respiration. Our analysis based on moving window SEM showed that substantial changes in

ecosystem respiration occurred when the water level crossed the tipping point of -24 cm. Before this critical point, our results showed a transition phase between -19 and -23 cm where shifts in plant and fungal communities progressively triggered shifts in ecosystem CO₂ respiration. Although the ultimate causality could not be established from SEMs, the joint consideration of the dynamic of ecosystem respiration and the successional trends in plant and

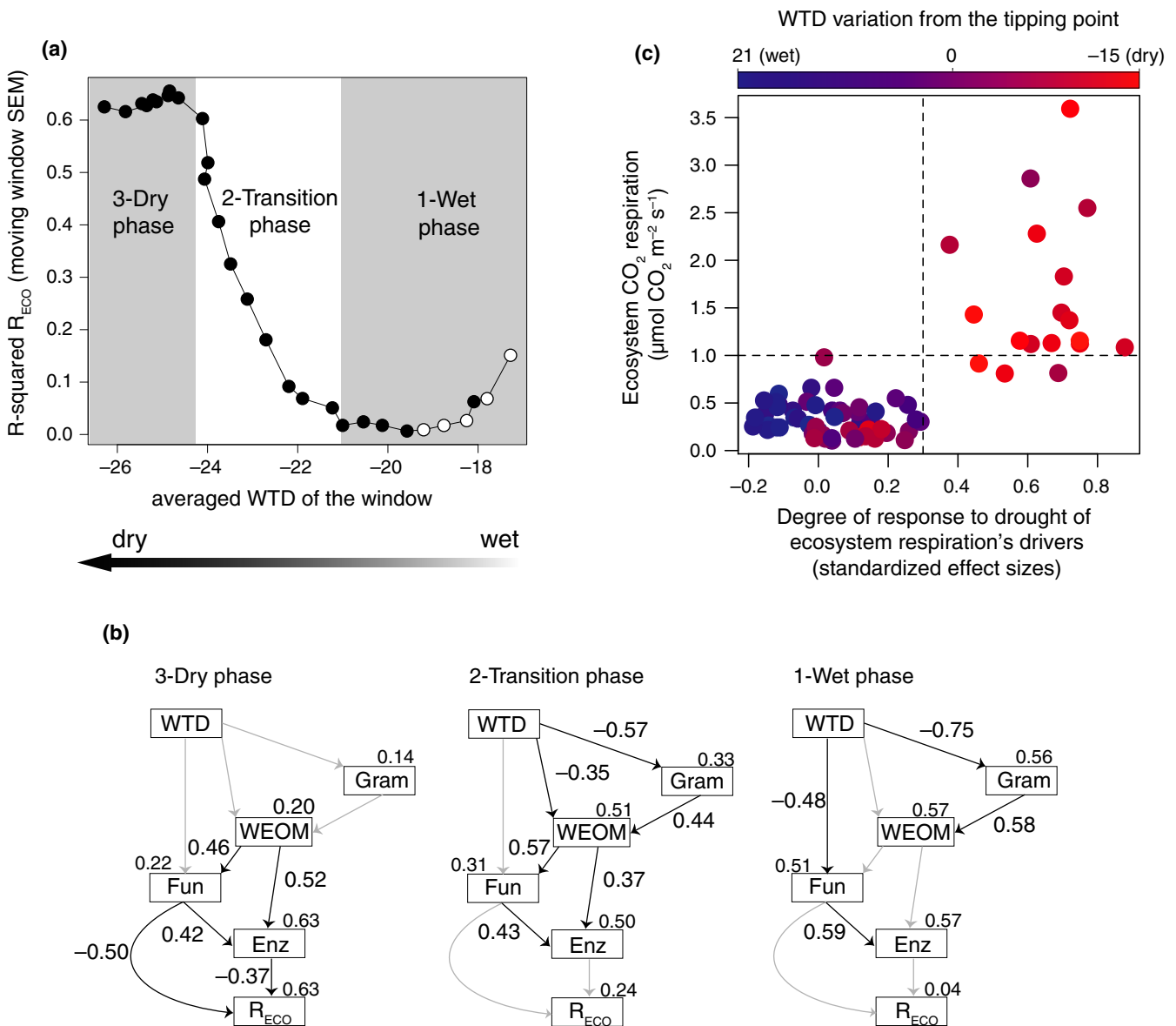


FIGURE 6 Moving window Structural Equation Modelling (SEM) evaluating the drivers of ecosystem CO₂ respiration. (a) Biplot showing the relationship between the response of ecosystem CO₂ respiration (R^2 -adjusted) by plant–fungal interactions in each SEM produced along the water level gradient (moving window SEM). Open symbols are SEMs with an unsatisfactory fit, filled symbols show the opposite. See Fig. S1 for the full a priori model considered. (b) Averaged moving window SEMs reflecting the main drivers of ecosystem CO₂ respiration for each of the three phases illustrated in (a). WTD = water level depth; Gram = graminoid cover; WEOM = quality of WEOM (spectral slope); Fun = fungal community composition; Enz = enzyme multifunctionality; R_{ECO} = ecosystem CO₂ respiration. Grey lines indicate nonsignificant paths. Only standardized path coefficients with $p < .05$ are shown. The amount of variance explained (R^2 , in italics) for each response variable are given on their respective box. All details on moving window SEMs are given in Table S6, including model fits. Note: the SEMs presented here were computed on the data set from 2013 only as fungi were not quantified in 2014. We, however, computed a similar model without fungi through the entire data set and found similar outputs (see Fig. S9). (c) Ecosystem CO₂ respiration plotted against the degree of response (standardized effect sizes) of its drivers. Dot colours represent the variation in water level from the tipping point -24 cm highlighted in moving window SEMs

fungal community structure along the water level gradient represents a major advance in understanding ecological thresholds (Briske et al., 2006; Kreyling, Jentsch, & Beier, 2014).

The impact of drought on ecosystem C dynamics involves multiple interactions among abiotic, plant and soil microbiota components (Scheffer et al., 2012; Suding & Hobbs, 2009). Our results demonstrate that simultaneous, and sometimes antagonistic, responses

within fungal and plant assemblages collectively pushes peatland processes beyond an alternative functional state. Consistent with previous studies (Asemaninejad, Thorn, & Lindo, 2017; Barnard et al., 2013; de Vries et al., 2012; Yuste et al., 2011), fungi displayed a marked resistance to drought and were even able to overcome the physiological stress imposed by decreasing water table depth. We observed greater fungal richness under dry conditions compared to

wet conditions, both over time and among water level treatments. Fungi are generally most competitive under an oxic environment (Bragazza, Bardgett, Mitchell, & Buttler, 2015; Jaatinen, Fritze, Laine, & Laiho, 2007), although water table depth was probably low enough (>−10 cm) at the beginning of the experiment to allow the oxygen diffusion through the peat surface (Estop-Aragónés, Knorr, & Blodau, 2012). Another reason for the fungal resistance to strong water level drop is the hydraulic redistribution of water by mycelia networks. Fungi are able to compensate unfavourable soil moisture conditions as long as water is available in other zones of the mycelia network (Guhr, Borken, Spohn, & Matzner, 2015), which was the case at the deeper water table level. Most importantly, we found that saprotrophic fungi gradually increased alongside with decreasing water level; they even predominated the fungal community composition once the water level passed −26 cm. This trend fits well with SEM outputs where enzyme multifunctionality regulates ecosystem CO₂ respiration. By producing a cocktail of extracellular enzymes (Crowther, Boddy, & Hefin Jones, 2012), saprotrophic fungi break down a wide range of organic compounds from simple and labile monomers to complex and recalcitrant polymers, which are rapidly consumed and largely respired.

Alongside with an increase in saprotrophic fungi, ecosystem CO₂ respiration was indirectly driven by drastic changes in vegetation composition. Graminoids gradually replaced *Sphagnum* mosses along the gradient with a parallel change in the interactions between plants and soil decomposers activity. Greater tolerance to drier conditions by graminoids compared to *Sphagnum* and shrubs is probably the result of anatomical and/or physiological traits of graminoids that protect them against drought-induced physiological damages (Carlquist, 2012). For example, their deep rooting system allows them to access water deeper in the peat when necessary (Buttler et al., 2015). We further observed that the spectrophotometric slope coefficient $S_{250-465}$ of WEOM, an index inversely related to the molecular weight and aromaticity of dissolved organic matter (Hansen et al., 2016), was greater under low water level and high graminoid cover. In accordance with other studies (Dieleman, Branfireun, & Lindo, 2017; Robroek et al., 2016; Wang, Richardson, & Ho, 2015), we interpret these results as evidence that the quality of WEOM was controlled by vascular plants, and especially by graminoids which released less aromatic C compounds presumably as root exudates (Crow & Wieder, 2005). Root exudates are indeed predominantly composed of simple labile C compounds, such as sugars (Gunina & Kuzyakov, 2015), easily available for microorganisms and easily respired as CO₂ (Werth & Kuzyakov, 2008). As fungi are particularly prone to use root exudates as energy source (Steinauer, Chatzinotas, & Eisenhauer, 2016), our results suggest that high graminoid cover stimulates fungal activity by providing labile C, thereby increasing the fungal respiration. An alternative or additional cause for the decreasing aromaticity of WEOM could result from higher peat oxygenation. Oxygen availability may stimulate oxygen-dependent enzyme activities (phenoloxidases and peroxidases) that decompose aromatic compounds such as polyphenols (Fenner & Freeman, 2011). However, oxidases did not increase in response to drier conditions

and were not involved in decreasing aromaticity of WEOM (Figs S7 and S10). Furthermore, we cannot exclude the possibility that living plants, especially graminoid plant roots and shrubs such as *Betula nana*, also contributed to increase ecosystem CO₂ respiration either directly or indirectly through plant–soil feedbacks (Deslippe, Hartmann, Grayston, Simard, & Mohn, 2016; Walker et al., 2016). Yet, previous findings showed that microbial heterotrophic respiration substantially contributes to ecosystem respiration in peatlands (~70%) (Dorrepaal et al., 2009). Although these findings do not exclude a contribution of plant root to ecosystem respiration in our plots, it suggests that the increase of ecosystem respiration was not primarily driven by autotrophic (=plant root) respiration.

Ecosystem CO₂ respiration was the greatest at the drier end of the water table gradient when graminoids and saprotrophic fungi became prevalent. We deduce that plant and fungal communities' responses to drought were large enough that an abrupt increase of ecosystem respiration was driven by the result of these changes, that is, persistent labile C inputs from graminoids that were degraded and respired by saprotrophic fungi. These results show that shift in ecosystem respiration critically depends not only on changing dominance within key trophic groups but also on their degree of response to drought. Moreover, we provide evidence that biotic communities are affected by extreme drought up to a point where changing dominance at one trophic level (tipping point in plants = −20 cm) causes a domino effect on other trophic levels (tipping point belowground ~ −24 cm) cascading into a systemic transition. Complex network modelling frameworks recently suggested that tipping points in the underlying interactions among species may be the main driver of ecosystem shifts (Scheffer et al., 2012; Yletyinen et al., 2016). Our findings extend these results by showing that sharp functional transitions in ecosystems may depend on the dominance replacement of sensitive by tolerant-specific species with the same function that together interact and drive specific ecosystem processes.

Our results have important implications for predicting the response of peatland C dynamic to drought by suggesting that *Sphagnum*-dominated peatlands with a water level lower than −24 cm may undergo a different functional state, with large contrasts in ecosystem CO₂ respiration. Recent studies showed that the current rate of climate change in northern biomes already shifted vegetation composition towards comparable changes to our study (Elmendorf et al., 2012; Lesica & Crone, 2017; Walker et al., 2006), with risks of substantive C loss (Bragazza et al., 2016; Ward et al., 2013). The range beyond which particular vegetation changes impact ecosystem functioning has been so far overlooked. In light of our findings, we suggest that droughts may turn peatlands into a C source once functionally interdependent shifts in plants and soil microbiota would become prevalent. Although we generalise from a limited set of ecosystem functions over a short-temporal scale, we cannot exclude that the system could follow a reverse pathway if the water level increases again (which is not currently the case, Fig. S11) or remains at the “dry stage” considering the legacy effects of drought on plant–soil interactions (Kaisermann, de Vries, Griffiths,

& Bardgett, 2017). Anyhow, we provide empirical understanding about the mechanisms pushing ecosystems beyond a threshold in response to climate change. The joint consideration of domino-like events within different trophic levels pave the way for detecting ecosystem shifts, and is an important step towards a truly mechanistic understanding that consider the nonlinear nature of ecosystem response to climate change.

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SUPPORTING INFORMATION

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