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Temperature and moisture effects on respiration in the organic horizon of a Pacific Northwest forest soil

Hanna M. (Hanna Maria) Winter
Western Washington University

Temperature and Moisture Effects on Respiration in the Organic Horizon of a Pacific Northwest Forest Soil

By Hanna M. Winter

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Chair, Dr. Peter Homann

Dr. Rebecca Bunn

Dr. David Hooper

MASTER'S THESIS

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A Thesis

Presented to

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In Partial Fulfillment

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Abstract

Ecosystem responses to temperature and moisture influence whether terrestrial ecosystems act as sources or sinks of atmospheric CO_2, an important greenhouse gas. Soil respiration—defined here as microbial release of CO_2 during decomposition—is a key process of CO_2 release to the atmosphere. This study focused on the response of soil respiration to temperature, moisture, and their interaction, and developed quantitative models to describe the nature of this interactive effect. This study addressed several under-examined components of the temperature and moisture effect on C and N dynamics: organic soils, multiple soil layers, a broad range of moistures, and a relevant, low-temperature range. Samples of upper and lower forest floor organic-horizon layers were collected from a mature conifer stand on the Olympic Peninsula in Washington State. Two experiments were conducted in which the soil samples were incubated at four temperatures (5.8–19.4°C) for up to four months, during which respiration was measured repeatedly. Because suboptimal moistures were expected to limit soil respiration, one experiment examined low soil moisture contents (<230% moisture) and the other high soil moisture contents (200–500% moisture). The lower organic layer had a lower C:N ratio and lower net N immobilization rate than the upper organic layer. The lower organic layer respiration rate was 25–45% of upper organic layer respiration at all temperature–moisture levels, but the response of the two layers to temperature and moisture was similar. Respiration increased with temperature under all moisture conditions, and Q_{10} ranged from 1.3 at 100% moisture to 1.9 at 220% moisture, and averaged 2.0 at higher moistures. Respiration demonstrated a saturating response to soil moisture. It increased an average of

1.4-fold as moisture increased from 100 to 220%, but was nearly constant at higher moistures. At the low moistures, the respiration response to moisture increased with increasing temperature. This respiration response was best described in regression models by a complex temperature–moisture interaction comprised of varying multiplicative effects. Long-term global C budget models, such as CENTURY and Rothamsted, currently assume a simple interactive effect comprised of a constant multiplicative effect of temperature and moisture on soil respiration; the models might be improved by inclusion of a more complex interaction such as that observed in this study. However, further studies are first needed to refine the equation(s) for describing this interaction, and to determine whether a single equation can be used to adequately describe soil respiration in all soils and soil layers.

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1.   Introduction

This study examined the effects of temperature, moisture and their interaction on soil respiration in an organic forest soil, and reviewed several different equations to describe these effects quantitatively. The temperature and moisture effects on soil respiration are of interest because soil respiration plays an important role in determining the direction of the net carbon (C) flux between terrestrial ecosystems and the atmosphere. Small increases in soil respiration rates due to climate change-induced variations in temperature and soil moisture may tip the C balance toward terrestrial ecosystems serving as a net source of C to the atmosphere.

1.1   The Big Picture: Global Carbon Cycle and Climate Change

Soils and terrestrial ecosystems are important components of the global C cycle, acting as both sinks and sources of C flux to the atmosphere. The earth contains approximately 10^8 Pg C (petagram = 10^{15} g; equal to gigatonne [Gt]) in the following pools: (1) the earth's crust (90,000,000 Pg), (2) oceans (38,000–40,300 Pg), (3) fossil fuels (4,000 Pg), (4) soils and terrestrial biosphere (2,050–2,160 Pg), and (5) the atmosphere (750 Pg) (Eswaran et al. 1993, Sundquist 1993). Typically, these C pools are kept in dynamic equilibrium by biogeochemical cycles, but anthropogenic activities have resulted in shifts of these C pools by 8.3 Pg C/year from fossil fuel burning and cement production, and 0.9 Pg C/year from land use changes (including deforestation) for a cumulative anthropogenic emission to the atmosphere of 555 Pg C since 1750 (IPCC 2013). Of this, 240 Pg C remain in the atmosphere, 155 Pg C have transferred to the ocean, and 160 Pg C have accumulated in

terrestrial ecosystems (IPCC 2013), especially in northern temperate latitudes (Tans et al. 1990, Sundquist 1993, Ciais et al. 1995). Carbon enters terrestrial ecosystems through photosynthesis and is released through autotrophic (shoot and root) respiration and heterotrophic (soil) respiration. Plant C is transferred to soils through litterfall, root turnover, and plant death, and this detrital C is then returned to the atmosphere through soil respiration (*i.e.,* heterotrophic respiration by soil microorganisms during organic matter decomposition).

Alteration of C fluxes, including soil respiration, in terrestrial ecosystems can have a major influence on C accumulation in the atmosphere. The combination of soil and root respiration is one of the major fluxes in the global C cycle, second only to gross primary productivity (*i.e.,* photosynthesis). Net Ecosystem Carbon Balance (NECB) determines whether terrestrial ecosystems act as net sources or sinks of C. NECB is defined as the net rate of C accumulation or loss in ecosystems, and is determined by the balance of C fluxes between gross primary productivity, autotrophic (root and plant) respiration, soil respiration, leaching, volatilization, and fire (Chapin et al. 2006). In most ecosystems, the largest component of NECB is net ecosystem productivity, the difference between net primary productivity (gross primary productivity less plant aboveground and root respiration) and heterotrophic respiration, primarily soil respiration. Estimates of these fluxes range from 68–100 Pg C/year for total soil and root respiration (Dobson et al. 1989, Raich and Schlesinger 1992) and 100–120 Pg C/year for gross primary productivity (Dobson et al. 1989, Houghton and Woodwell 1989).

Soil and root respiration account for the largest flux of C from ecosystems to the atmosphere (Ryan and Law 2005), and soil respiration may be more important than primary productivity as a control of ecosystem C exchange with the atmosphere (Valentini et al. 2000). Because of the size of these fluxes and similar rates at a global scale, relatively small changes in soil respiration rates may alter the balance between soil respiration and primary productivity, and lead to releases of stored C from ecosystems to the atmosphere (Schleser 1982, Raich and Schlesinger 1992, Rustad et al. 2000).

Increases in temperature and changes in precipitation patterns are predicted both globally and in the Pacific Northwest, and these could have large impacts on rates of soil respiration. The global average combined land and ocean surface temperature has thus far increased by 0.65–1.06°C since 1880, and is estimated to increase by 0.3–4.8°C by the end of the century, with greater warming over land than over the ocean (IPCC 2013). Averaged over the mid-latitude land areas of the Northern Hemisphere, precipitation has increased significantly since 1951, and climate change models predict increases in mean precipitation in many mid-latitude wet regions by the end of this century (IPCC 2013). Observed temperatures in the Pacific Northwest have increased by 0.2°C per decade since the 1950s, and are predicted to increase by 1.6–5.4°C by the 2080s (Mote et al. 2008). Projected precipitation changes are modest, but most models predict increases in winter precipitation and decreases in summer precipitation (Mote et al. 2008).

Biological and chemical processes, such as soil respiration and nitrogen (N) transformations, are strongly dependent on temperature (Hart and Perry 1999, Leiros et al.

1999, Rustad et al. 2001, Shaw and Harte 2001, Hart 2006, Van Meeteren et al. 2007).

Increased temperatures as a result of climate change may result in increasing microbial

activity and soil respiration, potentially contributing to a positive feedback to increased

atmospheric CO_2 concentrations. The increased atmospheric CO_2 concentrations leading to

increased global temperatures may result in increased soil respiration, which further

increases atmospheric CO_2 concentrations (Raich and Schlesinger 1992, Hart and Perry

1999, Rustad et al. 2000, Fissore et al. 2009, Bruijn and Butterbach-Bahl 2010, IPCC 2013).

Alternatively, if net primary productivity is more responsive to increased temperatures than

soil respiration, higher temperatures may spur increased plant growth, requiring additional

CO_2 uptake for photosynthesis and C sequestration in plant tissues, increasing terrestrial C

storage and helping to offset further CO_2 emissions in soils. Temperate, boreal and

tundra/alpine ecosystems hold more organic C than tropical ecosystems and may therefore

play a greater role in regulating or exacerbating the CO_2 feedback loop (Schleser 1982). Only

by understanding the response of soil respiration to increasing temperature and changing

moisture can the fate of C storage in terrestrial ecosystems, and the balance of plant

productivity and soil respiration, be better understood and predicted.

1.2   Soil Respiration: State of Knowledge

1.2.1   Temperature

Soil respiration increases with temperature (*e.g.,* Raich and Schlesinger 1992,

Howard and Howard 1993). Although a solid mechanistic understanding of how

temperature affects soil respiration is lacking, it is clear that, like other biochemical

reactions, respiration is strongly controlled by temperature. Low temperature limits

respiration through its effects on enzyme activity, including enzyme capacity, the affinity of

the enzyme for the substrate, and concentration gradients across cell membranes

(Davidson et al. 2006). Higher temperatures also change the forms of C that are available

for use by microbial communities (Waldrop and Firestone 2004). Despite the abundance of

studies examining the temperature dependence of soil respiration, no consensus on the

exact nature of this response has been determined; *i.e.,* no single equation has been found

that describes the temperature response in all soils investigated. Although the temperature

effect is typically described by an exponential function, the exact form of the model as well

as the parameter values have not been agreed upon. The main reason for this is that

confounding and feedback effects, particularly soil moisture and substrate availability, have

not been taken into consideration (Kirschbaum 2006). Several studies that examined only

the temperature response of soil respiration have suggested that moisture, as an additional

controlling factor, needs to be included in future research (Kirschbaum 2000, Rustad et al.

2001, Kirschbaum 2006).

1.2.2   *Moisture and Temperature–Moisture Interaction*

Moisture can limit soil respiration at low and high moisture levels. Low moistures

cause a decrease in microbial activity, including respiration, through the reduced diffusion

of soluble substrates, reduced microbial mobility, which leads to limited access to

substrates, and a direct effect on microbial growth (Grant and Rochette 1994). At very high

moistures, such as waterlogged soils or wetlands, microbial activity is limited by oxygen

deprivation due to reduced oxygen diffusion (Grant and Rochette 1994).

Many studies have identified significant effects of soil moisture on respiration rates

(Howard and Howard 1993, Azzalini and Diggle 1994, Shaver et al. 2006, Van Meeteren et

al. 2007, Chang et al. 2012, Guntinas et al. 2013), and several have found synergistic

interactive effects of moisture and temperature (Raich and Schlesinger 1992, Howard and

Howard 1993, Bowden et al. 1998, Leiros et al. 1999, Rey et al. 2005). While some studies

have found no significant effect of moisture on respiration rates (Edwards 1975, Howard

and Howard 1979, Fang and Moncrieff 2001, Waldrop and Firestone 2004), this is likely

because the moisture range examined was not sufficiently low or high to limit respiration.

Although most studies have found that temperature is a more important factor in

determining the rate of soil respiration than moisture, the addition of soil moisture

improves the predictive capability of models (Howard and Howard 1979, Raich and

Schlesinger 1992, Azzalini and Diggle 1994, Rey et al. 2005, Van Meeteren et al. 2007,

Klimek and Niklinska 2010, Guntinas et al. 2013), and at some sites with long dry periods,

moisture can act as the main factor influencing respiration rates (O'Connell 1990). In the

literature, the interactive effect of temperature and moisture on respiration is described as

a temperature-dependent moisture effect (Howard and Howard 1979, Bowden et al. 1998,

Rey et al. 2005) or a moisture-dependent temperature effect (Howard and Howard 1979,

Howard and Howard 1993, Shaver et al. 2006, Curiel Yuste et al. 2007, Van Meeteren et al.

2007, Klimek and Niklinska 2010, Guntinas et al. 2013), although these descriptions are simply different ways of describing the same synergistic interaction.

1.3   Nitrogen

Consideration of N dynamics in addition to C cycling is important when examining ecosystem response to global climate change because the N cycle may provide a link between C uptake by plants (net primary production) and C loss by soil respiration. The C and N cycles are coupled through C:N ratios of the vegetation and the soil organic matter pools, and fluxes between these pools (Shaver et al. 1992). The net C uptake of plants is limited by N, which is primarily provided by N mineralization of the soil organic matter, which is itself linked to soil respiration (organic matter decomposition) (Shaver et al. 1992). As plants have higher C:N ratios than the soil organic matter, the transfer of N from the soil organic matter (low C:N) to plants (high C:N) can increase the C storage of the system. The response of N mineralization to temperature and soil moisture may mitigate the increase in C loss from soil respiration if the mineralized N is taken up by plants that take in more C than is lost by stimulation of soil respiration (Shaver et al. 1992, Kirschbaum 2006). Conversely, increased litter production can result in N immobilization (*i.e.,* negative N mineralization), reducing plant productivity and net C intake (Kirschbaum 2006). In addition, N availability may directly influence soil organic matter decomposition or the forms of C available for use by microbial communities, and therefore C losses through soil respiration (Hobbie and Vitousek 2000, Waldrop and Firestone 2004).

Both C and N mineralization are stimulated by climate change-induced temperature and precipitation changes, but factors other than temperature and moisture may also affect N availability. Soil N mineralization rates and N availability are substantially controlled by temperature, and net N mineralization rates increase with increasing temperature (*e.g.,* Binkley et al. 1994, Peterjohn et al. 1994, Hart and Perry 1999). Moisture may also influence the rate of N transformations (Stevenson 1985, Leiros et al. 1999, Rustad et al. 2001), but perhaps to a lesser degree than temperature (Binkley et al. 1994, Van Meeteren et al. 2007). Interactive (synergistic) effects of moisture and temperature on N dynamics have also been observed (Binkley et al. 1994, Rustad et al. 2001). Other factors that influence N dynamics include season (Shaw and Harte 2001) and various measures of C availability, including organic matter content, the ratio of labile to recalcitrant organic matter, substrate quality, and/or C:N ratio of soil organic matter and surface litter (Stevenson 1986, Hart et al. 1994, Shaw and Harte 2001, Bruijn and Butterbach-Bahl 2010). N mineralization was influenced by vegetation type or biome in some studies (Binkley et al. 1994, Panikov 1999, Shaw and Harte 2001, Fissore 2009), but not in others (Rustad et al. 2001). Because of the potential influence of N on C cycling, it may be an important component to examine in conjunction with the temperature and moisture controls of soil respiration.

1.4   Modeling Soil Respiration

Despite the complexity of soil respiration controls, many studies describe the response of soil respiration with Q_{10} functions in which respiration responds exponentially to increasing temperature. The Q_{10} temperature coefficient is the ratio of the respiration

rate at temperature T+10°C to the respiration rate at temperature T. Q_{10} itself is temperature dependent, with higher Q_{10} coefficients at lower temperatures (Schleser 1982, Lloyd and Taylor 1994, Kirschbaum 1995, Fang and Moncreiff 2001, Klimek and Niklinska 2010), and may also depend on moisture (Curiel Yuste et al. 2007, Klimek and Niklinska 2010, Guntinas et al. 2013).

Temperature and moisture can influence respiration through additive, multiplicative, or complex effects. An additive effect occurs when an increase in temperature causes the respiration rate to increase by the same *absolute amount* at all moisture levels. With an additive effect, there is no interaction between the temperature and moisture. A multiplicative effect occurs when an increase in temperature causes the respiration rate to increase by the same *proportion* at all moisture levels. This indicates a simple interaction between temperature and moisture. A complex effect occurs when an increase in temperature causes the respiration rate to increase by different amounts and different proportions at different moisture levels. This indicates a complex interaction between moisture and temperature.

Thus far, no single equation has been found to describe the temperature and moisture dependence of soil respiration of all soils. Equations including only additive effects of temperature and moisture, multiplicative effects, and complex effects have been reported as the best equation to describe the effects of temperature and moisture on soil respiration in organic and mineral soils (see Appendix Table A9 for a summary of equations reported in the literature). Differences in the coefficients or form of the equation that best

describes the response of soil respiration may be due to factors other than temperature and moisture, including: organic soil C content (Kirschbaum 2000, Inclán et al. 2010, Chang et al. 2012); N content (Inclán et al. 2010, Chang et al. 2012); mineral vs. organic soils (Bowden et al. 1998, DeForest et al. 2009); substrate quality or age (Waldrop and Firestone 2004, Kirschbaum 2006, Shaver et al. 2006, Bruijn and Butterbach-Bahl 2010); elevation (Chang et al. 2012); C:N ratio (Bruijn and Butterbach-Bahl 2010, Klimek and Niklinska 2010); depth (Klimek and Niklinska 2010); or other soil-specific factors such as texture and pH (Parton et al. 1987, Kirschbaum 2000). Because climate models are projecting both increases in temperature and changes in precipitation, the nature and magnitude of interactive effects of temperature and moisture need further characterization to allow accurate predictions of how the C cycles of terrestrial ecosystems will respond to climate change.

Due to the potential implications of climate change on the global C cycle, many studies have attempted to model the response of C and N dynamics to climate change (*e.g.,* Parton et al. 1987, Jenkinson et al. 1991, Bruijn and Butterbach-Bahl 2010, Yurova et al. 2010). Many of these global and ecosystem-scale models include the response and feedback of C and N in soils because of the importance of soil respiration in the global C cycle. Two examples of ecosystem-scale models simulating soil C and N dynamics are the CENTURY model (Parton et al. 1987) and the Rothamsted model (Jenkinson et al. 1991). The CENTURY model simulates long-term (annual cycle over centuries or millennia) C, N, phosphorus, and sulfur dynamics through three organic matter fractions and two plant residue pools; the soil organic matter potential decomposition rate is reduced by a

multiplicative function of four driving variables: annual precipitation, temperature, soil texture and plant lignin content (Parton et al. 1987). The CENTURY model assumes that the majority of N is bound to C, and that therefore C and N fluxes are interactively linked (Parton et al. 1987). The Rothamsted C model simulates soil organic C decomposition at a decade to century timescale using five organic matter fractions whose maximum decomposition rates are modified by the multiplicative effect of temperature, soil moisture, plant cover and soil clay content (Jenkinson et al. 1991). The modifying factor of temperature is described as a logistic function, and the modifying factor of moisture (reported as topsoil moisture deficit) equals 1 (no modification) when moisture is not limiting at high moistures, decreases at moderate moistures, and remains low (at 0.2) at low moisture (high moisture deficit) (Coleman and Jenkinson 2008).

The respiration rates in the CENTURY and Rothamsted models involve two assumptions: (1) the response to temperature and moisture is a multiplicative effect, and (2) the responses to moisture and temperature are the same across all soil types (including both mineral and organic soils) and layers. To ensure that these models are as accurate as possible in describing C and N dynamics in soils today and in predicting responses to climate change decades from now, the underlying relationship of respiration to temperature, moisture and soil characteristics needs thorough examination to determine the validity of these assumptions.

1.5   Approaches to Evaluating Respiration

Soil respiration studies examining the effects of temperature and moisture are generally divided between two approaches: field studies and laboratory incubation studies. Both approaches have benefits and disadvantages, and both, as well as other approaches such as field warming experiments, are necessary to fill the gaps in the understanding of temperature and moisture controls on soil respiration.

Field studies are common and allow researchers to examine intact soils *in situ*. However, this approach does not readily allow for temperature and moisture manipulation, or direct measurement of respiration from the organic soil layer. Of particular concern is the confounding of temperature and moisture, particularly in areas with a warm dry summer and cold wet fall, winter and/or spring (Davidson et al. 1998, Kirschbaum 2000). For example, Davidson et al. (1998) found that respiration increased exponentially with soil moisture during drought conditions, but was negatively correlated with moisture during all other times of the year. The confounded effects of low temperature and high moisture during the fall, winter and spring seasons could not be separated to explain whether low respiration during these seasons was because of low temperature or high moisture, or a combination of the two (Davidson et al. 1998). Field researchers are also not able to control for other factors that may influence soil respiration other than temperature and moisture, such as fresh litter inputs (Edwards 1975, Kirschbaum 2000, 2006). In addition, Q_{10} obtained from field observation and field warming studies tends to be lower than Q_{10} for laboratory incubation studies, particularly at low temperatures, possibly because of the confounding

effects of moisture and substrate availability (Kirschbaum 2000, 2006). Unlike laboratory studies, however, field studies measure soil respiration as it actually occurs in the system under examination.

Several laboratory approaches have been used to examine the individual and interactive effects of temperature and moisture on soil respiration:

- Laboratory incubation of mineral soils, defined as soils or soil layers consisting predominantly of mineral matter and < 40% organic matter (Howard and Howard 1979, Howard and Howard 1993, Azzalini and Diggle 1994, Rey et al. 2005, Curiel Yuste et al. 2007, Sequaris et al. 2010, Chang et al. 2012, Guntinas et al. 2013)

- Laboratory incubation of plant litter or organic soil horizons, defined as soil layers with > 40% organic matter, primarily consisting of plant litter and/or partially decomposed litter lying above mineral soil layers (Edwards 1975, Howard and Howard 1979, O'Connell 1990, Bowden et al. 1998, Leiros et al. 1999, Shaver et al. 2006, Van Meeteren et al. 2007, Klimek and Niklinska 2010, Sequaris et al. 2010)

- Laboratory incubation of intact soil cores (Fang and Moncrieff 2001, Waldrop and Firestone 2004)

Laboratory incubation studies are valuable because they allow for manipulation of environmental factors, control over confounding effects, and direct observation of the

respiration response from organic soil layers. However, while laboratory incubation studies can provide a more controlled environment in which to examine temperature and moisture control of soil respiration than field studies, changes in the physical soil structure are inherent with soil sample collection and compositing, removal of roots, drying, sieving and other processing prior to commencement of the incubation study. The use of disturbed composite samples leads to a frequent problem encountered in laboratory incubation studies: a spike in the respiration rate at the beginning of the incubation (Fang and Moncrieff 2001). Laboratory incubation studies frequently use constant temperature, which does not take into account the diurnal cycle to which field soils are exposed. Long-term laboratory incubation studies can have problems with the exhaustion of labile organic matter, which results in fundamental differences in the characteristics of the soils under study, particularly if different incubation temperatures are used (Kirschbaum 2000). For these and other reasons, soil respiration measurements obtained in laboratory incubation studies are not scalable to predict soil respiration rates at the stand or ecosystem level.

Kirschbaum (2000, 2006) considers lab incubations the best and least biased approach for estimating the temperature dependence of organic matter decomposition, as the confounding factors of root respiration and substrate supply can more easily be controlled in a laboratory setting. However, the laboratory incubation of soil samples is fundamentally different from an intact soil pedon in the field, and it is unclear whether or how these artificial conditions may bias temperature sensitivity studies. Synthesis of the

results of all approaches remains the best way to obtain an overall consistent picture of the state of knowledge in the field.

1.6   Gaps in Knowledge

This study focused on several under-examined components of the temperature and moisture effect on C and N dynamics: organic soils, multiple soil layers, a broad range of moistures, and a representative, low temperature range. A summary of the need to fill these gaps follows:

Most studies examining the response of soil respiration to temperature and moisture have focused on laboratory incubation of mineral soils (*e.g.,* Guntinas et al. 2013, Zhou et al. 2014, and references cited therein) or field studies with natural or manipulated temperature and moisture regimes (*e.g.,* Poyatos et al. 2014, Zheng et al. 2014, and references cited therein). In comparison to the large number of studies on mineral soils, fewer researchers have examined organic soils or forest floor litter layers in a laboratory setting (*e.g.,* O'Connell 1990, Bowden et al. 1998, Leiros et al. 1999, Van Meeteren et al. 2007, Klimek and Niklinska 2010). Although they often have a much smaller C pool than mineral soils, organic soil and litter layers typically hold more labile C compounds and contribute significantly to total soil respiration. For example, in a field study, litter respiration accounted for nearly 50% of total soil respiration (litter layer and mineral layer combined) during most seasons, even though the litter C pool was only 6% of the mineral soil C pool (DeForest et al. 2009).

Different soil depths or soil layers may have differing respiration responses to temperature and moisture (Davidson et al. 1998, Klimek and Niklinska 2010). Different soil types (Howard and Howard 1993, Azzalini and Diggle 1994), mineral vs. organic soils (Bowden et al. 1998), and soils from different environments (*e.g.,* grassland vs. forest) (Fang and Moncrieff 2001, Rustad et al. 2001, Shaver et al. 2006) respond differently to moisture and temperature combinations, but few studies have examined variation among soil layers, especially for organic soils (Klimek and Niklinska 2010). In a lab incubation study, the lower organic layer was more sensitive to temperature changes than the upper organic layer, indicating that organic soil layer respiration rates respond differently to temperature and moisture changes (Klimek and Niklinska 2010). The depth at which soil temperature is measured in the field matters, suggesting that temperature dependence should be determined for each soil horizon, rather than for an arbitrarily chosen depth (Davidson et al. 1998).

Although respiration studies of forest soils are not uncommon, this type of research in temperate zones may further the understanding of the role of northern temperate terrestrial ecosystems as C sinks, currently responsible for accumulating nearly one-third of CO_2 emissions released from anthropogenic activities (Tans et al. 1990, Sundquist 1993, Ciais et al. 1995, IPCC 2013). As temperate, boreal, and tundra/alpine ecosystems hold more organic C than tropical ecosystems, they may play a greater role in regulating or exacerbating the CO_2 feedback loop (Schleser 1982). Projections of C flux due to climate change have predicted that soil organic C will increase in tropical zones and decrease in

boreal and tundra regions (Kirschbaum 2000). Furthermore, forest soil respiration responds more strongly to temperature increases than other ecosystem types, such as tundra or grassland (Rustad et al. 2001).

The use of a representative temperature and moisture range for the study site in question is important to accurately predict changes in soil respiration under climate change scenarios. Laboratory incubations frequently use temperatures that are greater than those actually experienced in the ecosystem under study. The problem with this is that fitting equations to respiration data for a range of temperatures including extremely high temperatures (often up to 35°C) can result in poor model fit at the low end of the temperature scale because of the bias in fitting the model to the higher temperatures (Fang and Moncrieff 2001). This can result in underestimation of the temperature effect at low temperatures, when soil respiration is more temperature sensitive than at high temperatures (Schleser 1982, Lloyd and Taylor 1994, Kirschbaum 1995, Fang and Moncreiff 2001, Klimek and Niklinska 2010). Some studies have concluded that moisture does not have an effect on respiration because the examined moisture ranges were not sufficiently low or high to limit respiration (Edwards 1975, Howard and Howard 1979, Fang and Moncrieff 2001, Waldrop and Firestone 2004). An ideal approach to understanding the effects of temperature and moisture on soil respiration involves examining a range of temperature and moisture that are actually present at the study site and are reasonably projected by climate models.

1.7   Objective and Research Questions

The objectives of this study are (i) to determine how soil respiration and N dynamics are influenced by temperature, moisture and their interaction in the organic horizon (O-horizon) of a Pacific Northwest forest, and (ii) to provide a statistical model of the response of O-horizon respiration to temperature and moisture. The research questions addressed in this study are:

- Under what conditions does moisture limit respiration?

- Does respiration response to moisture vary with temperature?

- Conversely, does respiration response to temperature vary with moisture?

- Does respiration response to moisture and temperature differ between O-horizon layers?

- Is the response of O-horizon respiration to temperature and moisture described by a single, unique statistical model?

- What is the response of net N mineralization to temperature and moisture?

- Does net N mineralization differ between O-horizon layers?

To address these questions, this study used laboratory incubation to assess the respiration rate and net N mineralization rate of two O-horizon layers from a conifer forest on the Olympic Peninsula in Washington State. The analysis and discussion of the effects of temperature and moisture and particularly the temperature–moisture interaction is

approached from several different perspectives in this study. These effects are described in

the literature as a temperature-dependent moisture effect, a moisture-dependent

temperature effect (Q_{10}), a saturating moisture effect, or by the use of models to

mathematically define the relationship. Although all perspectives are ultimately describing

the same interactive effect, a review of the multiple angles for describing the effect will be

covered in the discussion.

2.   Methods

2.1   Study Site

The study site is located on the Olympic Peninsula of Washington State, USA on Department of Natural Resources land near Sappho, WA. The site is the location of a long-term ecosystem productivity (LTEP) study examining the effects of biomass removal and woody debris on ecosystem productivity of early and late-successional forests in the Pacific Northwest. The site was railroad logged and burned in the 1940s, and is now an even-aged (~70 years) stand of naturally regenerated Douglas fir (*Pseudotsuga menziesii* [Mirb.] Franco), with some Sitka spruce (*Picea sitchensis* [Bong.] Carr.) and western hemlock (*Tsuga heterphylla* [Rafn] Sarg.) (Homann et al. 2008, U.S. Forest Service 2013). The site is flat, at an elevation of 100 m. The soil series is Solduc very gravelly sandy loam and the parent materials are loess and volcanic ash over glacial outwash (USDA Natural Resources Conservation Service 2014). The study site's climate is classified as dry summer variety oceanic (Kottek et al. 2006), which is defined as having warm summers and cool winters, with adequate precipitation for most of the year with the exception of a markedly drier summer. During the summer months between June and September, precipitation averages 8 cm per month and air temperature averages 15°C with a range of 8–23°C (Homann 2008, Homann personal communication 2014, PRISM Climate Group 2014). O-horizon moisture averages 178% between June and September, with 10% at <100% moisture, 59% at 100–200% moisture, 31% at >200% moisture (Homann personal communication 2014). Between October and May, precipitation ranges from 14–47 cm per month, O-horizon moistures

likely exceed 200%, and air temperature averages 7°C with a range of 1–17°C (Homann

2008, Homann personal communication 2014, PRISM Climate Group 2014).

2.2 Soil Sampling and Preparation

Upper and lower organic layer (O-layer) soils were collected from 10–20 sampling

locations in a ~1 ha area directly east of Sappho LTEP experimental unit #5 on April 24,

2012. The upper O-layer consisted of forest floor litter, primarily needles, while the lower O-

layer consisted of primarily undistinguishable decomposed organic matter and litter atop

the gravelly mineral soil. Samples were collected into 20–40 plastic bags, transported to the

lab in a cooler and stored at 4°C.

In the laboratory, the material collected was combined into single composite

samples – one for the upper O-layer and one for the lower O-layer. Water holding capacity

(WHC) was determined by saturating three replicate soil subsamples per layer, covered with

moist paper towel to prevent evaporation, and allowing the samples to drain overnight

(adapted from Rey et al. 2005). Subsamples were oven dried at 70°C to constant mass to

determine WHC (g water/g dry soil × 100%). Average WHC, 495% for the lower O-layer and

566% for the upper O-layer, was used to determine the highest moisture treatment level.

2.3 Experimental Design

Field-moist samples were dried by exposure to air to reduce moisture to

approximately 200%, and upper and lower O-layer samples were each divided among 32

incubation dishes with lids (approximately 42 g dry soil equivalent per dish). For each layer,

dishes were randomly assigned to 4 moisture levels crossed with 4 incubation chambers at

different temperatures, yielding 16 moisture–temperature combinations, with 2 replicate

dishes per combination. The moisture treatments were 200%,[1] 300%, 400%, and 500% (g

water/g dry soil × 100%). The incubation chamber temperatures ranged from approximately

5 to 20°C; actual average temperatures for the four incubation chambers were 5.8°C, 9.7°C,

14.8°C, and 19.4°C (see Appendix Table 1).[2] Soil incubation temperature was monitored

throughout the incubation period using two Thermochron iButtons (Dallas Semiconductor

Corp., Dallas, TX) per chamber. The iButtons were placed inside dishes similar to those used

in the respiration study.

2.4   Respiration

2.4.1   Study 1 – High Moisture

In Study 1, the high moisture study, moisture was initially adjusted to the assigned

moisture treatment by adding distilled water. During incubation, moisture was maintained

by adding distilled water every 2 to 3 days, as needed. Respiration was measured 11 times

during the 27-day incubation period using a Licor-6400 portable infrared gas analyzer fitted

with a soil respiration chamber attachment (Li-Cor, Lincoln, NE). On each measurement day,

three respiration measurements were taken. Lids were removed from the dishes at least 15

minutes prior to respiration measurement to allow trapped CO_2 to escape and to reduce

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[1] The lowest moisture treatment for the upper layer was approximately 230%. In analyses in which the upper layer is compared with the lower layer, the low moisture treatment for the upper layer is reported as 200%.
[2] Average measured temperature was used for all statistical analyses and in figures. However, for ease of reference, nominal temperature was used when temperature chambers were not directly compared, in tables and when discussing the incubation temperatures in the text.

"spikes" in the first respiration measurement. All respiration measurements were used to determine an average value, and average values were converted to $\mu molCO_2$/g remaining C/h. For the entire incubation period, a time-weighted average respiration was calculated for each dish.

2.4.2   Study 2 – Low Moisture Drying Study

During the high moisture study, it became clear that the soil moistures selected were not low enough to limit respiration; therefore, Study 2, the low moisture drying study, was developed to follow the high moisture study. In Study 2, dishes from the high moisture study were incubated at constant high moisture for 35 more days, then allowed to dry out for up to 66 days. Respiration measurements were taken periodically throughout the incubation period until respiration fell below detection limits (0.5 $\mu mol\ CO_2/m^2$/sec, equivalent to approximately 1.8 $\mu mol\ CO_2$/g C/h) (Gordon personal communication 2012). Lower O-layer dishes at 5, 10 and 15°C were excluded from this study because respiration rates were already near detection limits throughout the high moisture study. On each measurement day, two respiration measurements were made, averaged, and converted to $\mu molCO_2$/g remaining C/h.

2.5   Other Parameters

2.5.1   Initial Conditions

Initial pH and total C and N content of upper and lower O-layers were determined on six replicates (per layer) of composite soil samples. Distilled deionized water (50 ml) was

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added to 10 g soil for pH measurement. Solid-phase C and N were analyzed using a Thermo

NC 1112 Analyzer (Thermo Fisher Scientific, Waltham, MA).

2.5.2   Time

Although respiration changed with time throughout the two incubation periods,

temporal effects had a minimal effect on respiration in relation to the effect of temperature

and moisture. For the low moisture drying study, dishes at the same temperature and

different initial moistures reached 160% moisture at different times, but had similar

respiration rates at 160% moisture (Appendix Figure A4), suggesting time alone was not

influencing the decrease in respiration rate. See discussion in Appendix Section A2, Effect of

Time on Respiration, for details.

2.5.3   Inorganic N

Extractable nitrate (NO_3^-) and ammonium (NH_4^+) concentrations were measured for

pre-incubation samples and for soil samples collected from all dishes after the completion

of the high moisture study (on day 27 of the incubation period). The extractant used was

0.5M K_2SO_4, using a ratio of 1:20 (g dry soil to ml extractant). Samples were mixed and

filtered through Whatman 40 filters in Buchner funnels. Nitrate+nitrite and ammonia

concentrations in the extract were measured using a SmartChem 200 discrete analyzer

(Unity Scientific, Brookfield, CT) using Westco Scientific Instruments, Inc. (2007a, b)

methods for water, waste water, soil extracts and other aqueous samples (SmartChem 200

Method 375-100E-1 for nitrate-nitrite and SmartChem 200 Method 210-200B for ammonia).

2.6   Q$_{10}$ Calculations

For each moisture (100, 140, 180, 220% for low moisture study, and 200, 300, 400, 500% for high moisture study), the following equation was fit to the respiration rates:

$$\ln(Resp) = \ln(C_0) + \beta * Temp$$

Where ln(Resp) is the natural log of the respiration rate (μmolCO$_2$/g remaining C/h), ln(C$_0$) is the intercept (fitted parameter), β is the slope (fitted parameter), and Temp is temperature (°C). Q$_{10}$ was calculated from β as follows (Rey et al. 2005):

$$Q_{10} = e^{(10\beta)}$$

2.7   Statistical Analysis

Regression, one- and two-sample t-tests, and 1- and 2-factor ANOVA were used to determine effect, differences between temperatures, moistures, and layers, or to determine difference from zero slope (Table 1).

Table 1. Summary of statistical analyses in this study.

Layer	Test Type	Dependent Variable	Independent Variable
Regressions			
Upper, Lower	Linear Regression	Extractable Ammonium (after incubation)	Temperature (5.8, 9.7, 14.8, 19.4°C)
Upper (all temps), Lower (20°C only)	Linear Regression	Respiration	Moisture (200, 300, 400, 500%)

Table 1. Summary of statistical analyses in this study. (Continued)

Layer	Test Type	Dependent Variable	Independent Variable
Upper (low moisture and high moisture), Lower (high moisture only)	All possible regressions procedure	Respiration and ln-Respiration	Moisture, Temperature, Moisture×Temperature, Moisture-squared, Temperature-squared
Upper (low moisture and high moisture), Lower (high moisture only)	Akaike Information Criterion (AIC)	Respiration and ln-Respiration	Moisture, Temperature, Moisture × Temperature, Moisture-squared, Temperature-squared
Upper (low moisture and high moisture), Lower (high moisture only)	Linear Regression	Q_{10}	Moisture (four levels – for low moisture: 100, 140, 180, 220%; for high moisture: 200/230, 300, 400, 500%)

ANOVA and T-Tests

Layer	Test Type	Dependent Variable	Independent Variable
Upper vs. Lower	1-factor ANOVA	pH, total N, total C, C:N, initial extractable ammonium	Layer (upper, lower)
Upper, Lower (for each temperature individually)	2-factor ANOVA	Extractable Ammonium and Nitrate (after incubation)	Moisture (200, 300, 400, 500%) and Layer (upper, lower)
Upper, Lower	one-sample t-test comparing slope to zero (0)	Slope (for regression equation relating moisture to respiration for each temperature – layer combination)	
Upper vs. Lower (for 20°C only)	1-factor ANOVA	Intercept (for regression equation relating moisture to respiration)	Layer (upper, lower)
Upper vs. Lower (for 20°C only)	two-sample t-test	Slope (for regression equation relating moisture to respiration)	Layer (upper, lower)

Table 1. Summary of statistical analyses in this study. (Continued)

Layer	Test Type	Dependent Variable	Independent Variable
Upper, Lower (for each temperature individually)	2-factor ANOVA	Mean respiration	Layer (upper, lower) and Moisture (200, 300, 400, 500%)
Upper vs. Lower	1-factor ANOVA	Q_{10}	Layer (upper, lower)

I attempted to avoid or reduce pseudoreplication, the use of statistical tests without treatment replication or with replicates that are not statistically independent, in the statistical analysis of the data. This study does not include replication of temperature treatments, as four incubators each set to a different temperature were used – a common component of the experimental design of studies examining temperature controls of soil respiration in laboratory settings. Due to the lack of true replication of the temperature treatment, all moisture treatments for each temperature were grouped within one incubator (*i.e.,* the moisture treatments were not independent), which led to inability to directly assess the temperature–moisture interaction by using multiple regression or 3-factor ANOVA (factors: layer, temperature, moisture).

For analysis of the low moisture drying study, I ran a linear regression analysis to determine whether moisture had an effect on respiration for each dish (moistures below 230%) with at least three measurements. For each temperature, I calculated the average slope and intercept and the 95% confidence interval from the coefficients from the individual regression equations found for each dish. The averaged slope and intercept

represent the average response to moisture for the upper layer at a particular temperature. I ran a linear regression analysis to determine whether temperature had an effect on the average moisture–respiration slope (a measure of the moisture effect) to determine whether there was a temperature–moisture interaction.

To determine what equation(s) could be used to describe the temperature and moisture effects on soil respiration at low moistures, pseudoreplication was unavoidable as respiration data at multiple moistures at each temperature were required to examine the equations. However, pseudoreplication was reduced by finding 16 representative data points on which to model the effects. Each dish was randomly assigned to a moisture level (100, 140, 180, or 220%) within its moisture range, and the respiration rate at the assigned moisture was calculated from the previously determined regression equation for that dish. Replicates were averaged, yielding one average respiration rate for each of four moistures and each of four temperatures for the upper O-layer and for 20°C for the lower O-layer.

At high moistures, the effect of moisture on respiration was determined at each temperature individually, moistures were pooled and respiration rate averaged to determine the temperature effect. To model the response of respiration to moisture and temperature at high moistures, pseudoreplication was unavoidable, but reduced by averaging the moisture replicates and running the model comparisons using 16 representative data points (4 moistures x 4 temperatures).

Pseudoreplication was also avoided in Q_{10} analysis by use of an average respiration rate for each moisture and temperature combination. Q_{10} at each assigned (for low moistures) or initial (for high moistures) moisture level was determined as described above, and Q_{10} coefficients were compared using regression analysis of straight-line (n = 4) and logistic functions (n = 8).

α = 0.1 was used for all statistical tests. Due to small sample size, this α was chosen to increase power and to balance α and β error rates.

3. Results

3.1 O-Horizon Characterization

Compared with the upper O-layer, the lower O-layer was more acidic by 0.16 pH units (Table 2). The lower O-layer had 13% lower C concentration but 12% greater N concentration than the upper O-layer, resulting in a 22% lower C:N ratio.

Table 2. Summary of initial conditions for upper and lower O-layers.

Parameter	Upper O-Layer Mean (±95% CI)		Lower O-Layer Mean (±95% CI)		$P_{upper\ vs.\ lower}$*
pH	4.44	(0.052)	4.28	(0.1)	0.004
Nitrogen (g N/kg dry soil)	10.2	(1.8)	11.4	(1.59)	0.004
Carbon (g C/kg dry soil)	513	(25.8)	444	(82)	< 0.001
Carbon:Nitrogen	50	(7.81)	39	(2.88)	< 0.001
Ammonium (µg NH_3-N/g dry soil)	9.2	(5.56)	9.4	(5.65)	0.962
Nitrate (µg NO_3-N/g dry soil)	< 1.06		< 1.08		N/A

*1-Factor ANOVA (fixed factor = layer), n=6
Detection limits for nitrate were 1.06 µg NO3-N/g dry soil for the upper O-layer and 1.08 µg NO3-N/g dry soil for the lower O-layer
N/A = not applicable

Initial nitrate concentrations were below detection limits (< 1.06 µg NO_3-N/g dry soil for the upper O-layer and < 1.08 µg NO_3-N/g dry soil for the lower O-layer). Initial ammonium concentration was similar for both O-layers (Table 2). After 27 days of incubation, nitrate concentration remained below detection limits. During 27 days of incubation, the upper O-layer had lower net N mineralization rate than the lower O-layer at all temperatures (Figure 1). Neither layer x moisture interaction nor moisture had an effect

on the net N mineralization rate at any temperature (Appendix Table A8). Regression

analysis did not reveal a significant temperature effect on net N mineralization for either

layer (see Appendix Table A8).

Net N Mineralization by Temperature
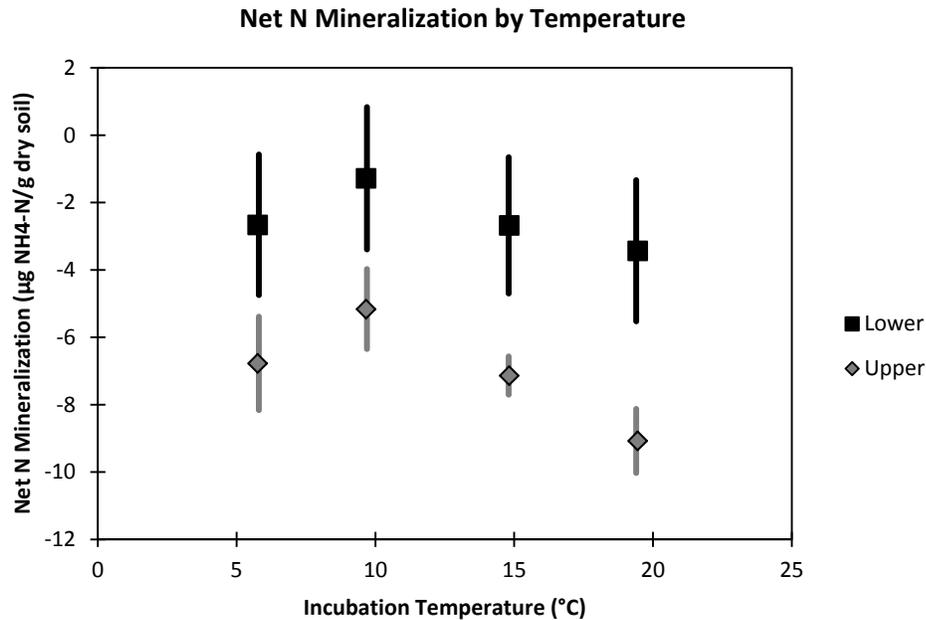
Figure 1. Net N mineralization (±95% confidence interval) during 27-day incubation for upper and lower O-layers at high moisture (200-500%). Each diamond and square represents the average of eight dishes (pooled across moisture). Two-factor ANOVA (4 moistures, 2 layers) at each temperature indicated no interaction and no moisture effect, but layers differed (P= 0.003 at 5°C, and <0.001 at other temperatures).

3.2   Respiration Response to Temperature and Moisture

To avoid pseudoreplication, the effect of moisture on soil respiration was

determined for each layer at each temperature individually. The moisture effect was then

compared among the four temperatures. See Section 2.7 Statistical Analysis for details.

In the upper O-layer, respiration rate increased as moisture increased from 100% to

230% (Figure 2A). This response increased with temperature (Figure 2A, Table 3); as

temperature increased, so did the slope of the regression line relating respiration to moisture:

$$\text{Moisture–Respiration Slope} = -0.862 + 0.193 \times \text{Temperature (°C)}$$

$$\text{Adj. } R^2 = 0.851, P = 0.051, n = 4 \text{ temperatures}$$

Above 230% moisture, moisture did not affect upper O-layer respiration rate (Figure 2B).

The lower O-layer respiration rates were approximately 25% of upper O-layer at lowest moisture (Figures 2A and 2C), and approximately 45% at higher moistures (Figures 2B and 2D, Table 4). The lower O-layer had a similar response to moisture and temperature as the upper O-layer (Figures 2C and 2D), although low respiration rates precluded assessment of the response to low moisture–low temperature combinations. The respiration–moisture slope at 20°C at low moistures did not differ between the two O-layers, but the y-intercept was 2.4 units higher for the upper O-layer (positive y-intercept) than the lower O-layer (negative y-intercept) (Table 3). At high moisture, only the lower O-layer at 20°C showed increased respiration with increased moisture (Figure 2D) (regression: $P = 0.026$, Adj. $R^2 = 0.52$, n = 8).
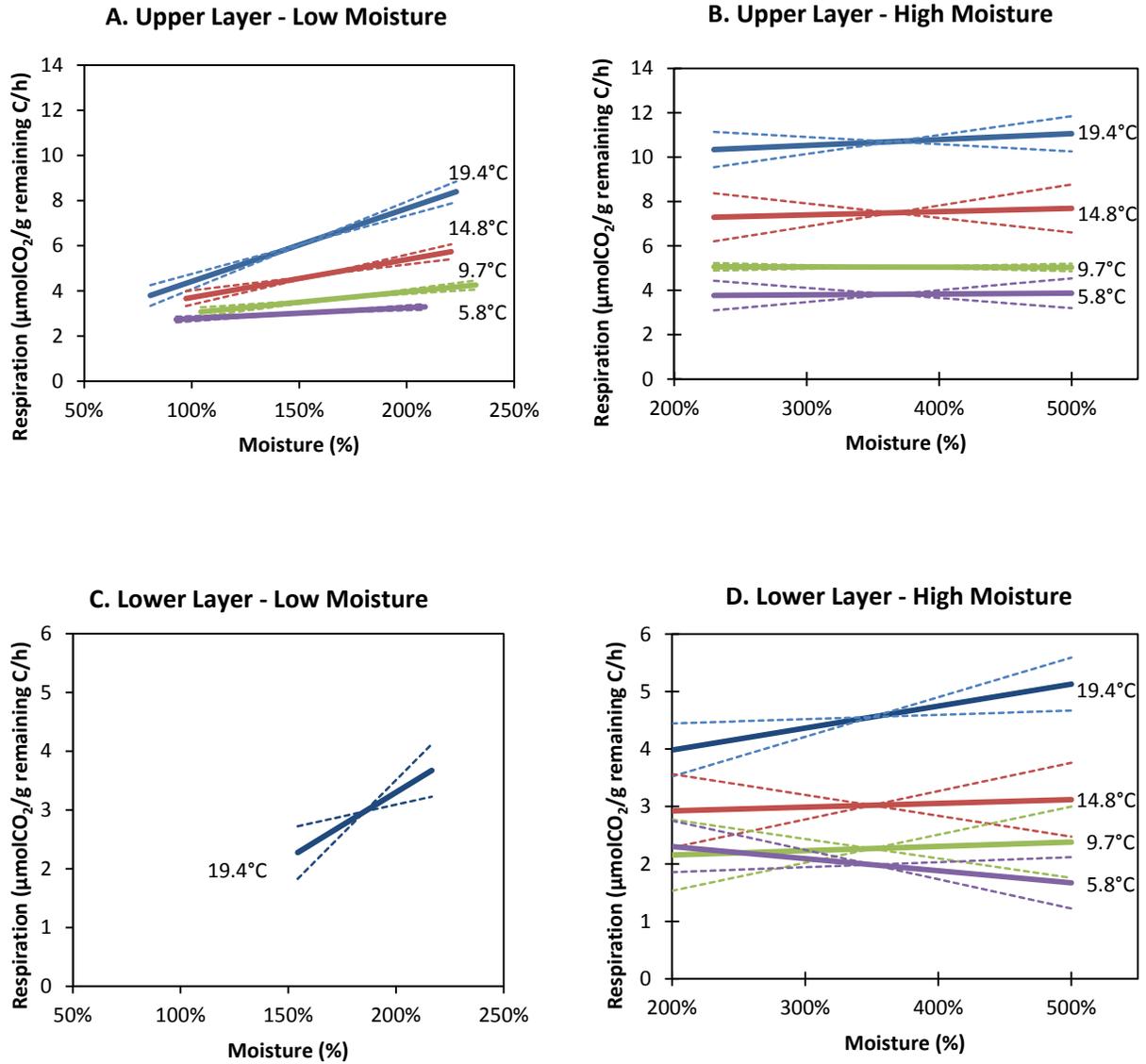
Figure 2. Average respiration (±95% confidence interval) by moisture for (A) low moistures (<230%) and (B) high moistures (230-500%) for the upper O-layer, and for (C) low moistures (<230%) at 20°C and (D) high moistures (200-500%) for the lower O-layer. n = 8 dishes per line for high moisture, n = 5 to 7 for low moistures, as listed in Table 3.

Table 3. Regression equations relating respiration rate to moisture in upper and lower O-layers at <230% moisture. Equations are of the form:

Respiration ($\mu molCO_2$/g remaining C/h) = a + b × Moisture (%)

Layer	Temp. (°C)	a (±95%CI)	b (±95%CI)	n	P_b*
Upper	5.8	2.3(0.45)	0.0048 (0.0022)	6	0.002
	9.7	2.1(0.55)	0.0093 (0.0031)	7	<0.001
	14.8	2.5(0.82)	0.015 (0.0058)	6	0.001
	19.4	1.2(0.67)	0.032 (0.0065)	6	<0.001
Lower	19.4	-1.2(2.5)	0.023 (0.014)	5	0.012
$P_{upper\ vs.\ lower}$**	19.4	0.023	0.10		

*$P_b$compares slope to 0, one-sample t-test

**$P_{upper\ vs.\ lower}$ compares equation coefficients for upper to lower O-layer at 19.4°C, two-sample t-test

Table 4. Respiration rate in upper and lower O-layers pooled across 200−500% moisture.

Temp. (°C)	Mean Respiration ($\mu molCO_2$/g remaining C/h) (±95%CI)		$P_{upper\ vs.\ lower}$*	n
	Upper Layer	Lower Layer		
5.8	3.8(0.40)	2.0(0.41)	<0.001	8
9.7	5.0 (0.34)	2.3 (0.86)	<0.001	8
14.8	7.5 (0.77)	3.0 (0.49)	<0.001	8
19.4	10.7 (0.73)	4.6 (0.54)	<0.001	8

*$P_{upper\ vs.\ lower}$ compares upper to lower mean at each temperature, two-factor (layer, moisture) ANOVA; moisture had no effect

3.3   Statistical Description of Temperature–Moisture Interaction

Complete avoidance of pseudoreplication was unavoidable for the statistical description of the temperature–moisture interaction. However, pseudoreplication was reduced by fitting models to a representative 16 point data set (4 moistures x 4 temperatures). See Section 2.7 Statistical Analysis for more details. Separate descriptions of the temperature–moisture interaction are provided for low moisture and high moisture here to allow for fitting and comparison of multiple equations to describe the interaction for the two different moisture ranges.

For the upper O-layer at moisture below 230%, the combined effects of temperature and moisture on ln-respiration were best described by a temperature term and multiplicative interaction term (Temperature × Moisture) (Table 5):

$$\ln(\text{Respiration as } \mu\text{mol } CO_2 \text{ / g remaining C/h}) =$$

$$0.829 + 0.0095 \text{ x Temperature (°C)} + 0.026 \text{ x Temperature (°C) x Moisture (\%)}$$

$$\text{Adj. } R^2 = 0.97, P < 0.001, n = 16$$

This equation and next four best equations, based on R^2 and AIC rank, were equations with complex interaction of temperature and moisture. The all-possible-regression procedure and AIC analysis agreed on the best equation and the top five equations, but disagreed on the order for equations ranked 2-5. Complete equation comparison results and coefficients are listed in Appendix Table A7.

Table 5. Best equation to describe temperature and moisture effects on ln(respiration) for upper layer at moisture < 230% and ranking based on regression (R^2) and AIC analysis. n = 16

Model	Adj. R^2	Rank (Adj. R^2)	Delta AIC(c)	Rank (ΔAIC[c])
Additive effect				
ln(Resp) = ln(a + bT + cM)	0.81	14	28.2	13
Multiplicative effect				
ln(Resp) = a + bT + cM	0.92	6	15.8	7
ln(Resp) = a + bT + cM + eT^2 + fM^2	0.91	10	23.4	11
Complex effect				
ln(Resp) = a + bT + dTM	0.97	1	0	1
ln(Resp) = a + cM + dTM	0.97	2	6.0	4
ln(Resp) = a + bT + cM + dTM	0.97	3	3.5	3
ln(Resp) = a + bT + cM + dTM + eT^2 + fM^2	0.97	4	12.1	5
ln(Resp) = a + dTM	0.96	5	1.8	2

Above 230% moisture, where moisture did not have an effect on upper O-layer respiration, ln-respiration was described simply by temperature (Table 6).

For the lower O-layer, low respiration rates precluded assessment of the response to low moisture–low temperature combinations. Although moisture had a significant effect on respiration at high moistures at 20°C for the lower O-layer, moisture did not have an effect on lower O-layer respiration at other temperatures, and the overall pattern for the lower O-layer ln-respiration was described simply by temperature (Table 6).

Table 6. Best equation to describe temperature and moisture effects on ln(respiration) for upper and lower O-layer at high moisture(200-500%) n = 16

Layer	Equation	p	Adj. R^2
Upper O-layer	ln(Respiration) = 0.89 + 0.076 x Temperature	< 0.001	0.977
Lower O-layer	ln(Respiration) = 0.26 + 0.061 x Temperature	< 0.001	0.820

3.4 Q_{10}

The average Q_{10} increased by 0.5 as moisture increased from 100 to 230% moisture for the upper O-layer (Figure 3A). Above 200% moisture, Q_{10} did not vary with moisture in either the upper or lower O-layer, and did not differ between the two layers (Figures 3B and 3C). This suggests that, at high moistures, the nature of the temperature effect does not depend on moisture or layer. Regression analysis found that a logistic equation described the relationship between moisture and Q_{10} across both low and high moistures of the upper layer, with a Q_{10} limit approaching 2.24:

$$Q_{10} = 1 / (1/2.24 + (1.10 \times 0.99^{\text{Moisture (\%)}}) \quad n = 8$$

Low respiration rates precluded assessment of the temperature response at low moisture for the lower O-layer.

A. Upper Layer - Low Moisture

$Q_{10} = 0.97 + 0.0044 \times$ Moisture
$P = 0.058$, Adj. $R^2 = 0.83$

B. Upper Layer - High Moisture

$P = 0.394$

C. Lower Layer - High Moisture

$P = 0.104$
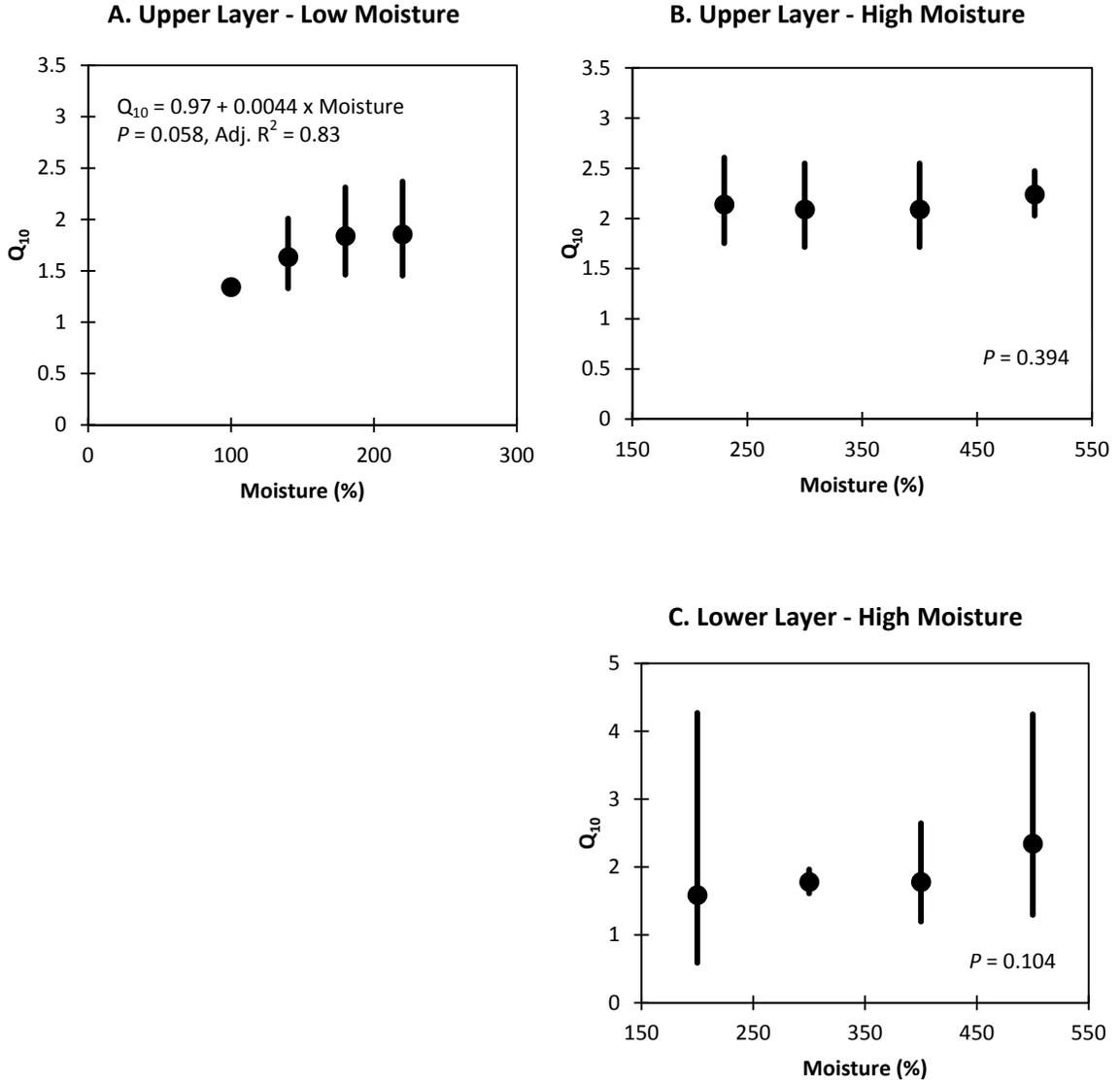
Figure 3. Q_{10} (± 95% confidence interval) for the upper O-layer at (A) low moistures (<230%) and (B) high moistures (230-500%), and (C) for the upper O-layer at high moistures (200-500%). 1-factor ANOVA (2 layers) found no difference between Q_{10} for upper and lower O-layers at high moisture: *P* = 0.16, n = 4 moistures.

4. Discussion

To understand and predict the fate of C in the global C cycle, and whether terrestrial ecosystems will become sources or sinks of C to the atmosphere, responses of C processes to environmental controls must be quantified. This study focused on the response of soil respiration to temperature, moisture and their interaction, and attempted to find a model or models to describe the nature of this interactive effect. I found a complex interactive effect of temperature and moisture on soil respiration. Moisture had a saturating effect on soil respiration: at low moistures the moisture effect increased with temperature, while at high moisture, there was no significant effect of moisture. Moisture also had a saturating effect on Q_{10}; the multiplicative temperature effect (Q_{10}) increased with moisture at low moistures, but moisture did not have an effect on Q_{10} at high moistures. The upper O-layer had a higher respiration rate than the lower O-layer at all temperature and moisture treatment levels, but the qualitative response of respiration of the two layers to temperature and moisture did not differ. Net N immobilization was not significantly influenced by either temperature or moisture, but was greater for the upper O-layer than the lower O-layer.

The analysis and discussion of the effects of temperature and moisture and particularly the temperature–moisture interaction are approached from several different perspectives in this study. These effects are described in the literature as a temperature-dependent moisture effect, a moisture-dependent temperature effect (Q_{10}), a saturating moisture effect, or by the use of models to mathematically define the relationship.

Although all perspectives ultimately describe the same interactive effect, a review of the multiple angles for describing the effect will be covered in the discussion.

4.1   O-Horizon Characterization

Total C and N contents of the soils in this study were 50% greater than previously measured average values at this site. Previous studies have found 301 g C/kg soil and 6.5 g N/kg soil at this study site (Homann et al. 2008). Total N content was within the typical range for Pacific Northwest O-horizon forest soils (3.5-19.5 g N/kg soil) (Gessel et al. 1973). The C:N ratio, 39 for the lower O-layer and 50 for the upper O-layer, are similar to the C:N ratio of 46 found by Homann et al. (2008).

Inorganic N was primarily in the form of ammonium, and net N mineralization rates were negative, denoting net N immobilization in both soil layers. The upper O-layer had a 65% greater net N immobilization rate than the lower O-layer. The C:N ratio explains the negative net N mineralization rate (net N immobilization) and the difference in net N mineralization rate between the two layers. Both layers had relatively high C:N ratios, but the upper O-layer had a higher C:N ratio than the lower O-layer. The C:N ratio, and therefore the N content of the soil organic matter, is often a strong driver regulating the release and uptake of inorganic N, with lower C:N ratios (higher total N) associated with greater releases of inorganic N (Stevenson 1986, Killham 1994). In soils with higher C:N ratios, microbial N requirements are higher than what is present in the organic matter, and inorganic N mineralized by decomposition is immediately taken up by the microbes (Mengel

1982, Stevenson 1986). Net N immobilization, as found for both O-horizon layers, occurs when available mineral N is readily taken up by microbes because N demand exceeds the rate of N mineralization (Stevenson 1986). The relatively higher net N immobilization rate in the upper layer is therefore explained by the higher C:N ratio, as compared to the lower layer.

Net N mineralization during 27 days of incubation was not significantly influenced by temperature or moisture for either layer. Other studies have found contrary results. Some studies have found a positive relationship between N mineralization and soil moisture or precipitation (Binkley et al. 1994, Leiros et al. 1999, Rustad et al. 2001), while others have found a negative relationship (Van Meeteren et al. 2007). Temperature responses have been more consistent, with a positive correlation between N mineralization and temperature (Binkley et al. 1994, Leiros et al. 1999, Rustad et al. 2001, Van Meeteren et al. 2007). Net N mineralization response to temperature is similar in mineral and organic soils (Peterjohn et al. 1994, Leiros et al. 1999), while the moisture effect is stronger in organic soils than in mineral soil (Leiros et al. 1999).The nature of the net N mineralization response to climatic factors is likely more complex than organic matter decomposition and soil respiration (Kirschbaum 2000). Since net N mineralization is the change in inorganic N over time, it is the balance of two processes: gross N mineralization and microbial immobilization. Both of these processes are affected by temperature and moisture, and the relative responses of gross mineralization and immobilization to climactic factors will determine the effect on net N mineralization. Net N mineralization may be less temperature

sensitive than organic matter decomposition, and the temperature effect on net N

mineralization is not as strongly influenced by temperature at low temperatures as organic

matter decomposition (Kirschbaum 1995).

4.2   Moisture Effect on Respiration — Limits

I found a saturating response of respiration to soil moisture: at low moistures,

respiration increased with increasing moisture, and at high moisture, respiration was not

affected by change in moisture. The finding that respiration increased with increasing

moisture up to 230% in this study agrees with the findings of several studies that have

found that moisture effects are only important to respiration rates at the low end of the

moisture spectrum (O'Connell 1990, Davidson et al. 1998, DeForest et al. 2009, Inclán et al.

2010). Low soil moistures decrease soil respiration by limiting microbial activity – at low

moisture, soluble substrate diffusion and microbial mobility are reduced, leading to limited

access to substrates (Grant and Rochette 1994). This, combined with reduced microbial

growth, results in low respiration rates (Grant and Rochette 1994). As soil moisture

increases, microbial activity is no longer suppressed by moisture limitation, leading to no

effect of moisture on respiration rates at high moistures. O'Connell (1990) found that

respiration increased with increasing moisture up to 100%, above which no further

moisture effect was observed. They developed a logistic statistical model to describe the

effects of temperature and moisture on litter respiration rates, including a saturating

moisture effect. Similarly, several field studies have found that moisture is an important

predictor of soil respiration, but only in the dry period or during drought conditions when soil moistures are low (Davidson et al. 1998, DeForest et al. 2009, Inclán et al. 2010).

Moisture limitation of respiration occurs at both low and very high moistures (Howard and Howard 1993, Bowden et al. 1998, Shaver et al. 2006). At very high moistures, such as waterlogged soils or wetlands, microbial activity is limited by oxygen deprivation due to reduced oxygen diffusion (Grant and Rochette 1994). This leads to reduced respiration rates when moisture is increased from "moist" to "wet" conditions (Shaver et al. 2006). Some studies have found that log-respiration was best described by a quadratic equation that described moisture limitation both at low and high soil moisture for organic forest floor soils (Bowden et al. 1998) and mineral soils (Howard and Howard 1993). Other researchers have observed that models based on temperature alone overestimated respiration at very low and very high moistures, indicating that moisture limitation may play a role at the extremes, but found no significant effect of moisture on soil and litter respiration (Edwards 1975). Although no moisture effect was observed at the highest soil moisture treatments in this study, this does not preclude moisture limitation at moistures greater than water holding capacity (~500%) for these soils.

The range of moisture treatments selected for any particular respiration study can affect the findings and obscure potential moisture effects. Several studies have found no effect of moisture on soil respiration, including this study at high moistures (200-500%), except for the lower O-layer at 20°C (Edwards 1975, Howard and Howard 1979, Mathes and Schriefer 1985, Fang and Moncrieff 2001, Waldrop and Firestone 2004). A possible reason

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for no observed moisture effect may be that only moderate moisture levels were used, and that these levels were not high or low enough to limit respiration (Fang and Moncrieff 2001, Waldrop and Firestone 2004). Indeed, the results of the low moisture drying study show that the moisture range initially selected for the high moisture study (200-500%) was not low enough to limit respiration, and incapable of revealing the moisture effect and moisture–temperature interaction observed in the low moisture drying study. Conversely, some studies have found increases in respiration throughout the moisture range examined (Leiros et al. 1999, Van Meeteren et al. 2007). Here, again, this effect may be the result of a limited range of moistures examined; this time, relatively low moistures (below the optimal moisture range for respiration) may result in the appearance of a constant moisture effect across the full range of moisture levels chosen.

The results of this study and others examining both organic and mineral soils (Raich and Schlesinger 1992, Rey et al. 2005, Van Meeteren et al. 2007, Guntinas et al. 2013) suggest that moisture has a saturating effect on soil respiration. This is especially important at sites with long periods of low soil moisture, during which moisture is the main factor limiting soil respiration (O'Connell 1990).

4.3   Moisture Effect on Respiration — Variation with Temperature

In addition to the saturating effect of moisture alone, this study confirms that the moisture effect on O-horizon respiration is greater at higher temperatures (Figure 2). The temperature-dependence of the moisture effect is one approach to describe the complex

interactive effect of temperature and moisture on soil respiration. Other researchers have also described the interaction as a temperature-dependent moisture effect in which the moisture effect is magnified by temperature, both in litter (Bowden et al. 1998) and mineral soils (Howard and Howard 1979, Howard and Howard 1993, Rey et al. 2005). This temperature–moisture interaction can be explained by the synergy of the individual effects of temperature and moisture on soil respiration. When low temperatures limit enzyme activity, increased substrate availability provided by increased soil moisture can only marginally improve conditions for microbial activity and soil respiration. When soils are warm (20°C), moisture increases spur microbial growth and substrate availability, increasing soil respiration.

4.4   Temperature Effect on Respiration — Q_{10}

I found an exponential response of respiration to increased temperature. Q_{10} temperature coefficients ranged from 1.3 to 2.3 (Figure 3). These coefficients are within the broad range of Q_{10} coefficients found in similar studies of organic soils: 1.28 to 3.67 (Klimek and Niklinska 2010), 2.03 (Bowden et al. 1998), and 2.62 (Leiros et al. 1999). A synthesis of several field studies across the globe found that average Q_{10} was 2.4, with a range of 1.3 to 3.3 (Raich and Schlesinger 1992). In a synthesis of mineral soil laboratory incubation studies, average Q_{10} ranged from approximately 6 at 5°C to 2.5 at 20°C (Kirschbaum 2006). Q_{10} coefficients at low moistures for this study are lower than the traditionally cited Van't Hoff rule of thumb of Q_{10} of 2 to 3 for chemical reactions (Howard and Howard 1993), although

the average Q_{10} at high moisture for this study was 2.0, which is at the low end of this range.

Despite its frequent usage in soil respiration studies, several authors have discussed the downsides of using Q_{10} to describe the temperature effect. Not only may Q_{10} be site specific, Q_{10} is higher at lower temperatures (Schleser 1982, Kirschbaum 1995, Davidson et al. 1998, Fang and Moncreiff 2001, Klimek and Niklinska 2010) and depends on moisture (this study, Curiel Yuste et al. 2007, Klimek and Niklinska 2010, Guntinas et al. 2013). The Q_{10} for a particular soil can range from 2.2 to 8.8 depending on the equation used to calculate it (Fang and Moncrieff 2001). The Arrhenius equation, which is commonly used to calculate Q_{10}, may underestimate the temperature response at low temperatures (Fang and Moncrieff 2001). This may lead to underestimation of CO_2 releases from soils when temperatures increase in cold climates or during the winter. Although the Arrhenius model provides a good theoretical description of a single chemical reaction, it is not appropriate for describing the combined response of various chemical, physical and biological processes (Kirschbaum 2006). Ultimately, Q_{10} in isolation is insufficient in describing the response of soil respiration to climate change because it does not take into account other factors, such as moisture, which also influence respiration rates.

4.5   Temperature Effect on Respiration — Variation with Moisture

The temperature–moisture interaction can also be described as a moisture-dependent temperature effect. I found that moisture has a saturating effect on the

temperature effect (Q_{10}) in which Q_{10} increased with increasing moisture from 100% to

220% moisture, beyond which moisture no longer limited respiration; increased moisture in

excess of 230% did not affect Q_{10}. The varying Q_{10} across the moisture spectrum represents

a complex interaction of temperature and moisture. The moisture dependence of the

temperature effect at low moistures found in this study has also been observed in a

laboratory incubation of mineral soil cores (Curiel Yuste et al. 2007). However, other studies

of organic soils have not reported a saturating effect of moisture on Q_{10}. I found that the

relationship of Q_{10} to moisture was described by a logistic equation with a limit approaching

2.24. The equation models the synergistic effect of temperature and moisture at the low

end of the moisture spectrum, with the temperature effect (Q_{10}) increasing with increasing

moisture, and the saturation of the moisture effect on Q_{10} at 300% moisture and above,

where moisture does not have an effect on Q_{10}. This two-phased effect – complex

interaction at low moistures, with effect saturation at high moistures – can be explained by

the synergistic effects of temperature and moisture. When low moistures limit substrate

availability and microbial growth, increases in temperature that would otherwise spur

enzyme activity can only marginally improve conditions for microbial activity and soil

respiration. When soil moisture is not limiting (>230% moisture in this study), temperature

alone limits the respiration rate.

However, others have found the opposite effect of moisture on the temperature

effect; namely, that Q_{10} is higher at low moistures than at high moistures. This conflicting

result has been found in incubations studies of both organic (Klimek and Niklinska 2010)

and mineral soils (Guntinas et al. 2013). An incubation study of organic soils found a temperature effect only at low moisture (50%), while no change in soil respiration was found with a temperature increase from 5 to 15°C at moderate to high moistures (100 and 200%) (Van Meeteren et al. 2007). In another study, at the upper end of the moisture spectrum, the temperature effect was smaller at high moistures ("wet" treatment) than at moderate moistures ("moist" treatment), suggesting that high moisture can dampen the temperature effect (Shaver et al. 2006). At high moistures, oxygen depletion can occur due to slow diffusion rates, significantly decreasing soil respiration by aerobes (Grant and Rochette 1994). Other than the waterlogged soil (Shaver et al. 2006), the studies that found a decreasing temperature effect (Q_{10}) with increasing moisture do not suggest a possible mechanism behind these patterns, but it is unlikely that very high (waterlogged) soil moistures were the cause of this result because these studies still found an increase in soil respiration with increasing moisture (Klimek and Niklinska 2010, Guntinas et al. 2013). Finally, a mineral soil incubation study found that all soil moistures showed the same temperature response despite a significant moisture effect and temperature–moisture interaction (Rey et al. 2005). These varying responses of the temperature effect to moisture may be due to other factors. Chang et al. (2012) found that total N was positively correlated with mineral soil respiration and Q_{10}, and that the correlation was stronger at higher temperatures and moistures. Although this effect was not directly examined in this study, this study suggests the opposite effect. The upper O-layer, which had lower total N than the lower O-layer, had a higher respiration rate than the lower O-layer. This indicates that, if total N has an effect on respiration rates, higher total N appears to be connected with

lower, not higher, respiration rates for the soils in this study. In addition, the temperature

response (Q_{10}) was not different between the two layers, indicating that total N likely did

not influence the temperature response in these soils.

4.6   **Difference in Respiration Response Between Layers**

Consistently higher respiration rates for the upper O-layer than for the lower O-layer

can be explained by the difference in C:N ratio. Higher respiration rates from upper-layer

soils as compared to deeper horizons were also found in a study of organic forest soils in a

Polish spruce forest (Klimek and Niklinska 2010) and in mineral Mediterranean forest soils

(Rey et al. 2005). Soil respiration rates may be partially controlled by C:N ratio (Bruijn and

Butterbach-Bahl 2010, Klimek and Niklinska 2010). Indeed, C:N ratio was higher for the

upper O-layer in both this study and in a similar study (Klimek and Niklinska 2010), which

had higher respiration rates than the lower O-layer. When respiration at 20°C and moderate

moisture is plotted against C:N ratio for this and similar studies (Bowden 1998, Leiros et al.

1999, Van Meeteren et al. 2007, Klimek and Niklinska 2010), respiration generally increases

with increasing C:N ratio (linear regression: P = 0.088, Adj. R^2 = 0.448, n = 6). Soils with

higher C:N ratios (such as upper O-layers) not only contain more C, but this C is likely more

labile than in lower horizons or for soils with lower C:N ratios. Once the labile organic

matter is respired, more recalcitrant organic matter is left behind and incorporated into

lower horizons, resulting in a decrease in substrate quality with increased depth (Rey et al.

2005, Klimek and Niklinska 2010).

Despite different overall respiration rates, the two O-horizon layers responded similarly to temperature and moisture. I found that neither Q_{10} at high moisture nor the moisture effect at 20°C differed between the two O-horizon layers. Other studies show conflicting results – a study of organic soils found a decrease in Q_{10} with depth (Klimek and Niklinska 2010) while a study of mineral soils found an increase in Q_{10} with depth, but no significant differences in the temperature sensitivity coefficient used to calculate Q_{10} (Rey et al. 2005). Differences in Q_{10} with depth may indicate a difference in temperature sensitivity between labile and recalcitrant organic matter fractions (Rey et al. 2005). The conflicting results from these studies may be due to differences in litter type (Howard and Howard 1979), soil type (Howard and Howard 1993), ecosystem (Shaver et al. 2006, Chang et al. 2012), or chemical quality or decomposability of the soil organic matter, or other differences in soil chemistry and physics (Shaver et al. 2006).

Contradictory results are reported in the few studies that have compared the temperature and moisture sensitivity of organic and mineral soil respiration. Compared to mineral soil, litter respiration had lower apparent temperature sensitivity but higher sensitivity to soil moisture in one study (DeForest et al. 2009). Conversely, another found that forest floor organic soil respiration was more responsive to temperature and moisture than mineral soils (Bowden et al. 1998). Differences in the temperature and moisture responses of soil respiration in mineral and organic soils may be due to differences in substrate availability (DeForest et al. 2009) or quality (Shaver et al. 2006). Yet another study found that although mineral soil respiration was more sensitive to temperature changes

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than organic soil respiration, the interaction coefficients in the regression equations found for the organic and mineral layers were similar, suggesting that the moisture–temperature interaction has a similar effect on both organic and mineral soil layers (Leiros et al. 1999).

4.7   Statistical Models of Moisture and Temperature Effects on Respiration

A complex temperature–moisture interaction best models the effects of temperature and moisture on soil respiration at lower moistures. The effect of temperature and moisture on soil respiration for the upper O-layer at moistures below 230% was best described by an equation with a temperature term and a temperature–moisture interaction term. Although this was the best equation for this study site, several other equations also worked well in describing the temperature–moisture interaction, but the equations modeling a complex interaction worked better than equations modeling a multiplicative interaction, and both multiplicative and complex interactions were better than equations with no interaction of temperature and moisture. Several other studies have also found a complex interaction between temperature and moisture (Raich and Schlesinger 1992, Bowden et al. 1998, Leiros et al. 1999), some have found multiplicative interaction (Davidson et al. 1998, DeForest et al. 2009, Klimek and Niklinska 2010), while yet others have found only additive effects of temperature and moisture with no temperature–moisture interaction (Van Meeteren et al. 2007, Chang et al. 2012). An equation with no temperature–moisture interaction term to predict log-respiration models a multiplicative interaction, because log transformation of the dependent variable takes into account the interaction of moisture and temperature without requiring a separate interaction term

(Howard and Howard 1993, Rey et al. 2005). Upon inspection of the additive and multiplicative effects, complicated equations sometimes only model a multiplicative interaction (*e.g.,* O'Connell 1990, DeForest et al. 2009). For a summary of temperature and moisture effects found in the literature, see Appendix Table A9.

As several equations could be adequately used to predict respiration from temperature and moisture for this study, I wanted to know how models reported by other studies compared to the results of this study. To compare the results of this study to similar studies of organic soil respiration (O'Connell 1990, Bowden et al. 1998, Leiros et al. 1999, Van Meeteren et al. 2007, hereafter the "comparison studies"), the equations found in the comparison studies were fitted to the data from this study (upper O-layer, low moisture). The coefficients in the equations from the comparison studies were converted to match the units used in this study ($\mu molCO_2$/g [remaining] C/h). As Klimek and Niklinska (2010), who studied two organic forest soil layers from a spruce forest in Poland, did not provide an equation to describe the temperature and moisture interaction, estimates of their data were extracted from their Figure 1B, and equations were fitted to the estimates to allow for comparison to other studies, as appropriate.

All comparison studies reported an increase in respiration with temperature, and moisture had a strong influence at low moistures (Figure 4). However, there were quantitative differences between the comparison studies and this study (Table 8). A fundamental difference between the studies must be noted – different moisture ranges were used by the comparison studies. Van Meeteren et al. (2007) and Leiros et al. (1999)

used only low moistures, similar to the ones in the low moisture drying study portion of this

research, reflecting the portion of the moisture range that is limiting to respiration. Bowden

et al. (1998) and O'Connell (1990) used a broader range of moistures, including both low

and high moistures, which required the modeling of both moisture limitation and a range of

moistures that did not affect respiration. The Bowden et al. (1998) equation was fitted to

the data from this study at low moistures as well as across the full range of moistures

examined. The O'Connell (1990) equation was only fitted to the data from this study across

the full range of moistures.

A. Van Meeteren et al. 2007

Present Study 20 ---
Van Meeteren 20 ———
Van Meeteren 5 ———
Present Study 5 ---

$Resp = 1.0 + 0.16T + 0.0097M$

B. Leiros et al. 1999

Present Study 20 ---
Present Study 5 ---
Leiros 20 ———
Leiros 5 ———

$Resp = -0.091 + 0.070T - 0.0028M + 0.00069TM$

C. Bowden et al. 1998

Bowden 20 ———
Klimek/Niklinska 20 ·····
Bowden 5 ———
Klimek/Niklinska 5 ·····

$\ln(Resp) = -0.13 + 0.0080T + 0.0028M + 6.0E\text{-}5T^2 - 6.6E\text{-}6M^2 - 6.9E\text{-}6TM$

D. O'Connell 1998

Present Study 20 ---
O'Connell Jarrah 20 ———
Present Study 5 ---
O'Connell Jarrah 5 ———

$Resp = (\exp(-2.26 + 0.263(1 - 0.00397T/0.263)) / (1 + 25.8e(-0.094M))$

Figure 4. Comparison of regression equations fitted to data from (A, B, D) this study or (C) Klimek and Niklinska (2010) with original regression equations from similar studies (A) Van Meeteren et al. 2007, (B) Leiros et al. 1999, (C) Bowden et al. 1998, and (D) O'Connell 1998. Lines show respiration at temperatures of 5 and 20°C across a range of moistures (%). Stated equations are from the similar studies.

Table 7. Comparison of this study to five similar studies, Van Meeteren et al. 2007, Leiros et al. 1999, Bowden et al. 1998, Klimek and Niklinska 2010, and O'Connell 1990, as well as CENTURY-type equation, and the best equation found for the present study.

Study	Soil	Equation (converted to Resp units: $\mu molCO_2/g$ C/h) significance* (±95% CI for coefficients)	Temp Range (°C)	Moisture Range (%)	Adj. R^2	P	SE of the Est.†	Delta AIC(c) and rank ††
Resp=T+M								
Van Meeteren et al. 2007	Litter	Resp = 1.0 + 0.16T+0.0097M	5-20	50-200	0.59	<0.001	N/A	N/A
This Study	Upper O-horizon	Resp = -0.89 + 0.23***T + 0.015***M (1.4)(0.062)(0.0071)	5-20	100-220	0.85	<0.001	0.60	28.2 (13)
Resp=T+M+TM								
Leiros et al. 1999	O-horizon	Resp = -0.091 + 0.070T − 0.0028M + 0.00069TM	4-25	40-220	0.75	<0.001	N/A	N/A
This Study	Upper O-horizon	Resp = 3.0*** − 0.079T − 0.0089*M + 0.0020***TM (1.8)(0.13) (0.011) (0.00079)	5-20	100-220	0.95	<0.001	0.34	17.4 (8)

Table 7. Comparison of this study to five similar studies, Van Meeteren et al. 2007, Leiros et al. 1999, Bowden et al. 1998, Klimek and Niklinska 2010, and O'Connell 1990, as well as CENTURY-type equation, and the best equation found for the present study.

Study	Soil	Equation (converted to Resp units: μmolCO$_2$/g C/h) significance* (±95% CI for coefficients)	Temp Range (°C)	Moisture Range (%)	Adj. R^2	P	SE of the Est.†	Delta AIC(c) and rank ††
ln(Resp)=T+M+TM+T^2+M^2								
Bowden et al. 1998	Forest floor	ln(Resp) = -0.13 + 0.0080**T + 0.0028**M + 0.000060T^2 - 6.6E-6**M^2 - 6.9E-6TM	5-25	64-322	0.89	<0.001	N/A	N/A
This Study	Upper O-horizon	ln(Resp) = 0.84** - 0.0062T + 0.0011M + 0.00051T^2 − 4.32E-6M^2+ 0.00027***TM (0.59) (0.046) (0.0065) (0.0016) (0.000020) (0.00014)	5-20	100-220	0.97	<0.001	0.059	12.1 (5)
This Study	Upper O-horizon	ln(Resp) = 1.47 − 0.25T + 0.020**M + 0.019**T^2 − 4.0E-5***M^2+ 0.00080***TM (2.55) (0.34) (0.011) (0.013) (1.7E-5) (0.00037)	5-20	100-500	0.94	<0.001	0.66	N/A
Klimek and Niklinska 2010	Olf/Ofh	ln(Resp) = 0.46* + 0.011**T + 0.00032**M - 0.00012T^2 - 3.6E-7M^2 - 3.2E-6*TM (0.043) (0.0045) (0.00019) (0.00012) (2.6E-7) (3.8E-6)	5-30	75-600	0.87	0.005	0.011	N/A

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Table 7. Comparison of this study to five similar studies, Van Meeteren et al. 2007, Leiros et al. 1999, Bowden et al. 1998, Klimek and Niklinska 2010, and O'Connell 1990, as well as CENTURY-type equation, and the best equation found for the present study.

Study	Soil	Equation (converted to Resp units: $\mu molCO_2/g$ C/h) significance* (±95% CI for coefficients)	Temp Range (°C)	Moisture Range (%)	Adj. R^2	*P*	SE of the Est.†	Delta AIC(c) and rank ††
Resp=(e^(α + βT (1+γT/β))/ (1 + δe^(ΘM))								
O'Connell 1990	Jarrah litter	Resp= (exp (-2.26 + 0.263(1 − 0.00397T/0.263) / (1 + 25.8exp(-0.094M))	4-40	10-260	0.932	N/A	N/A	N/A
O'Connell 1990	Karri litter	Resp = (exp (-1.16 + 0.215T(1 − 0.00318T/0.215) / (1 + 23.1exp(-0.07M)	4-40	10-260	0.943	N/A	N/A	N/A
This Study	Upper O-horizon	Resp = (exp (0.524 + 0.0469T(1 + 0.00097T/0.0469) / (1 + 4.34exp(-0.0167M)	5-20	100-500	0.92	N/A	N/A	N/A
Resp = max * [a*e^(bT)] * [c + dM] (CENTURY-type model; Parton et al. 1987)								
This Study	Upper O-horizon	Resp = 19.99 (0.126e$^{(0.0574T)}$)(0.328 + 0.00306M)	5-20	100-220	0.930	N/A	N/A	33.4 (14)
ln(Resp) = a + bT + dTM (best equation from the present study)								
This Study	Upper O-horizon	ln(Resp) = 0.829 + 0.0095T + 0.026TM (0.075) (0.0094) (0.0047)	5-20	100-220	0.971	<0.001	0.053	0.0 (1)

* indicates *P* < 0.1; ** indicates *P*< 0.05; *** indicates *P*< 0.01

†The standard error of the estimate is the square root of the residual mean square, which is an estimate of the average squared error in prediction

†† Note that AIC analysis ranking is based on comparison of all models for temperature and moisture effects on respiration at low moisture (see Appendix Table A7). AIC analysis excludes the O'Connell model and the Bowden model for the full moisture range because these models used a larger moisture range than the other models, and therefore cannot be directly compared using AIC analysis. AIC score and rank for models with units as "Resp" are based on equivalent equation converted to "ln(Resp)"to maintain common response variable for accurate comparison.

N/A = data not available

Van Meeteren et al. 2007: shrub heathland in the Netherlands; litter equation for 0-48 day incubation; did not indicate significance of individual equation coefficients

Leiros et al. 1999: oak forest in Spain; did not indicate significance of individual equation coefficients

Bowden et al. 1998: mixed hardwood forest in Massachusetts, USA; specified which individual equation coefficients were significant (*P* < 0.05)

Klimek and Niklinska 2010: spruce forest in Poland, equations calculated from data extracted from their Figure 1B

O'Connell 1990: eucalyptus forest in Australia; did not indicate significance of individual equation coefficients

This Study: Douglas fir forest in Pacific Northwest Washington, USA

Although the model includes no temperature–moisture interaction term, there is remarkable agreement between Van Meeteren et al. (2007) and the current study, both in the response of respiration to moisture and temperature, as well as the magnitude of respiration (Figure 4A). The similarity in respiration response in a heathland in the Netherlands (Van Meeteren et al. 2007) and a conifer forest in the Pacific Northwest (the current study) is confirmed by similar temperature and moisture coefficients. However, since this equation does not include any temperature–moisture interaction, other equations better describe the respiration response of the soils from the current study, as evidenced by the low AIC ranking (13 out of 17).

The addition of a temperature–moisture interaction term to the Van Meeteren et al. (2007) equation produces the equation of Leiros et al. (1999) and greatly improves the model fit (Figure 4B). This simple addition changes an equation without any interaction into an equation that models a complex interaction. However, the moderate AIC ranking (8 out of 17) indicates other equations better describe the respiration response of the current study. Leiros et al. (1999) and the current study had similar sign and magnitude of the moisture and temperature–moisture interaction coefficients, although the temperature term was not significant for the current study. The magnitude of respiration for the current study was greater than for Leiros et al. (1999), and is reflected by the greater (positive) intercept for the current study. The difference in vegetation type – oak woodland in Spain (Leiros et al. 1999) vs. conifer forest in the Pacific Northwest (the current study) – and related factors such as organic matter quality (Waldrop and Firestone 2004, Kirschbaum

2006, Shaver et al. 2006, Bruijn and Butterbach-Bahl 2010) may explain the difference in intercept. Raich and Schlesinger (1992) also found that a similar equation with additive and multiplicative temperature and moisture (as precipitation) terms best predicted global soil respiration rates.

Bowden et al. (1998) and Klimek and Niklinska (2010) found that respiration increased with moisture to a point, then decreased with additional moisture. Bowden et al. (1998) modeled this effect with a quadratic equation including both additive effects of incubation temperature and soil moisture as well as an interaction term – a complex interaction. Klimek and Niklinska (2010) did not provide an equation, but reported that respiration increased with increasing moisture (from low to moderate moisture), then decreased with additional increase in moisture (from moderate to high moisture), suggesting a curvilinear effect. The quadratic equation significantly fit all studies compared (Bowden et al. 1998, Klimek and Niklinska 2010, and the current study), however, not all terms in the model were significant. Only the temperature term, moisture term and moisture-squared term were significant for Bowden et al. (1998). For Klimek and Niklinska (2010), only the intercept, temperature term and moisture term were significant. For the current study, only the intercept and the temperature–moisture interaction term were significant. The fact that neither quadratic term (temperature-squared nor moisture-squared) was significant for the current study or Klimek and Niklinska (2010) suggests that the quadratic terms are not needed to explain the variation in respiration for these soils. However, AIC analysis ranked this equation as the fifth best model overall, indicating that

even though not all terms are significant, it is still a good model for predicting the response

of respiration to temperature and moisture. When the quadratic equation was fitted to the

full moisture spectrum, the quadratic terms were significant, although the intercept and

temperature terms were not. The lack of a quadratic response for Klimek and Niklinska

(2010) may be explained by the fact that the moisture effect was only apparent when

respiration was pooled across temperatures; the effect is not apparent when temperature

and moisture are considered separately. The magnitude of respiration for the current study

was much higher than for the other studies, and was therefore excluded from Figure 4C.

O'Connell (1990) and the synthesized results of this study (full moisture range)

found a positive moisture effect at low moisture and no moisture effect at high moisture

that could be modeled by a logistic function (Figure 4D). O'Connell (1990) found that

respiration increased with temperature and with moisture up to 100%, above which no

further moisture effect was observed. Similarly, the current study found that respiration

increased with moisture from 100–230%, but no increase in respiration was observed when

moisture increased from 230–500%. The moisture response was much stronger and more

rapid (steeper increase) for O'Connell (1990) than for the current study; respiration

increased by a factor of 11 from the lowest to the highest moisture for O'Connell (1990),

but only by a factor of 1.8 for the current study. In addition, the respiration plateau at high

moisture increased by a factor of 11.6 between 5 and 20°C for O'Connell (1990), but only by

a factor of 3.0 for the current study. Although the model reported by O'Connell (1990)

appears more complicated than the other equations reported in this comparison, it only

models a multiplicative interactive effect of temperature and moisture. This illustrates the difficulty in incorporating the complex interaction between temperature and moisture that occurs in nature. The current study had a higher magnitude of respiration than O'Connell (1990) at both temperatures, which, as described above, may be described by differences in vegetation (eucalypt forest vs. conifer forest) or soil organic matter quality.

Ultimately, a variety of different equations can be used to adequately describe the response of respiration to temperature and moisture, but the inclusion of a temperature–moisture interaction term is necessary, and a complex interaction model is best. Despite the variety of equations found by similar studies and the differences in coefficients, the data from this study were fit by each of the equations found in the comparison studies. The two best comparison equations ($\ln[\text{Resp}] = a + bT + cM + dTM + eT^2 + fM^2$ [Bowden et al. 1998] and $\text{Resp} = a + bT + cM + dTM$ [Leiros et al. 1999]), which were ranked 5th ($R^2 = 0.97$) and 8th ($R^2 = 0.95$) in AIC analysis, respectively, both described a complex interaction of temperature and moisture. The all-possible-regressions procedure further found that a wide variety of traditional linear regression equation types worked well in predicting respiration from temperature and moisture, but the complex interaction was best at describing this relationship, as evidenced by the top ten AIC-ranked equations describing a complex interaction (Appendix Table A7). A complex interaction is the best way to describe the temperature–moisture interaction, but a variety of equation forms that include a complex interaction can be used to predict respiration from temperature and moisture; the five best equations, based on AIC and regression, had R^2 of 0.96–0.97, suggesting similar

ability to predict respiration. However, the ability to use multiple equations to describe the

respiration response to temperature and moisture for soils other than those examined in

this study is unknown, as multiple equations were not reported for the similar studies used

in this comparison. As previously mentioned, the moisture range selected for a particular

study affects the type of equation that can be used to describe the data. It is clear from the

comparisons above that respiration in a study that uses a broad moisture range (such as

O'Connell 1990) cannot be described by a simple linear equation.

4.8   Comparison with Functions in Process Models

Addition of the complex interactive effect of temperature and moisture on soil

respiration can improve ecosystem-level C budget models. The CENTURY and Rothamsted

models simulate ecosystem C budgets and calculate soil respiration rates, but they assume

only a multiplicative effect of temperature and moisture (Figures 6A and 6B). The ratio of

respiration at 20°C to respiration at 5°C remains static across all moistures: 6.0 for CENTURY

and 5.6 for Rothamsted. In contrast, the findings of this study indicate the temperature

response varies with moisture; *i.e.,* the interaction of temperature and moisture is complex.

For the upper O-layer in this study, the ratio of respiration at 20°C to respiration at 5°C

ranged from 1.5 at 100% moisture to 2.2 at 220% moisture, and averaged 1.8 at higher

moistures. An equation similar to the temperature and moisture functions used in modeling

respiration response in CENTURY was fitted to the results of this study for the upper O-layer

at low moisture, but this equation explained only 93% of the variation in the respiration

response, and was ranked 14th out of 17 equation types compared in AIC analysis (Figure 5c,

Table 8, Appendix Table A7). This and other studies that have found complex temperature–

moisture interactions provide a basis to evaluate and possibly improve respiration functions

in C budget models. Ultimately, the complex interaction may need to be taken into account

in global modeling of soil C.
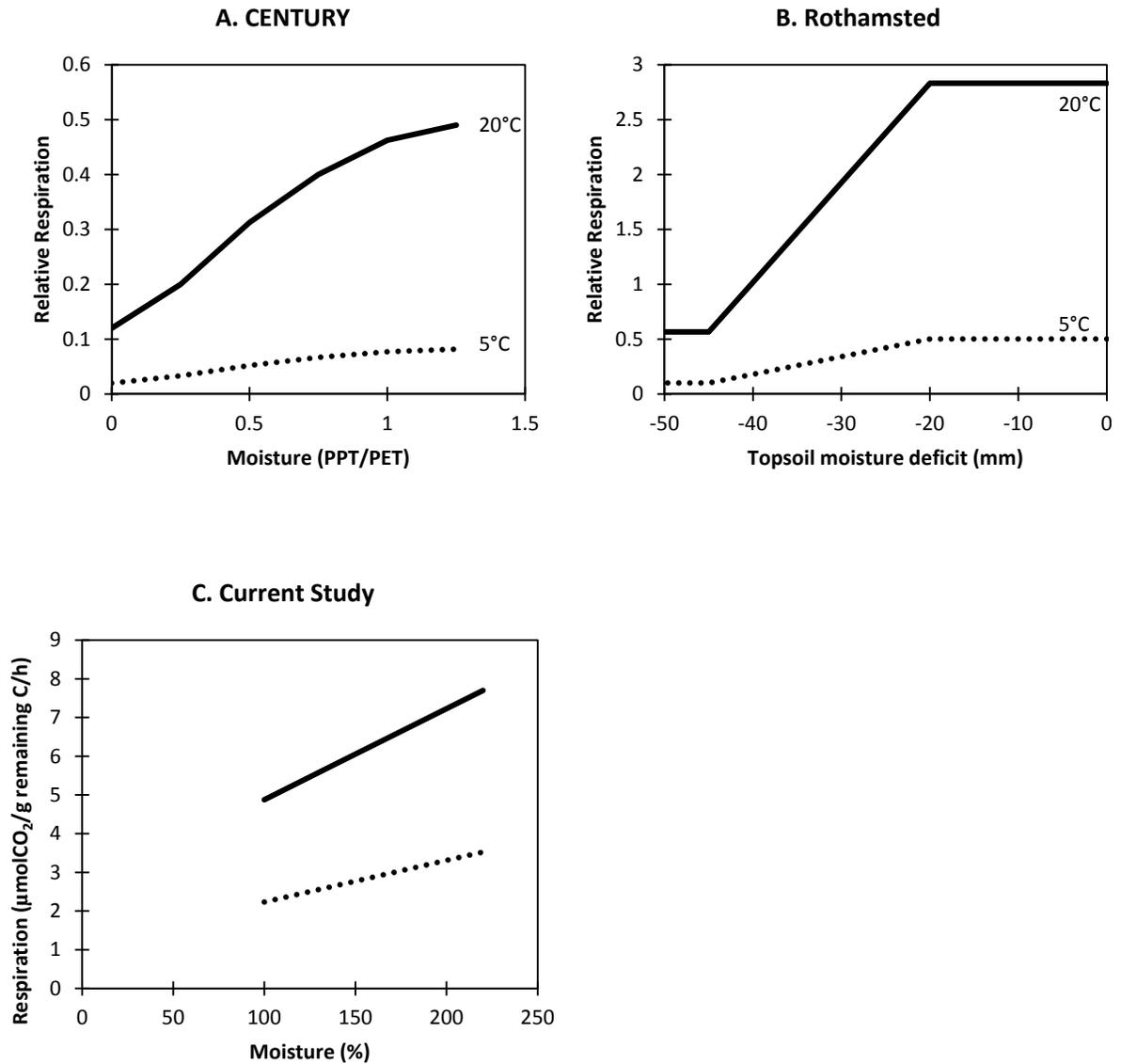
Figure 5. Relative respiration by moisture for (A) CENTURY model (Parton et al. 1987), (B) Rothamsted model (Coleman and Jenkinson 2008), and (C) CENTURY-type equation fitted to data from this study, upper O-layer at low moisture.

4.9   Future Study Recommendations

Additional studies of the respiration response of organic soils are needed to describe

the temperature and moisture controls of soil respiration and their interactions

comprehensively, particularly to refine the form of the equation that models this interaction. Future studies should consider the following:

- Reduce and/or acknowledge pseudoreplication;

- Address the potential confounding effects of time;

- Use a broad spectrum of moisture and temperature treatments over the natural range for the study site to capture the full spectrum of potential moisture and temperature–moisture effects on soil respiration, and to further refine the nature and limits of the temperature–moisture interaction;

- Report moisture units as gravimetric water content (g water/g dry soil), water holding capacity, and matric potential whenever possible to allow for better comparison of the results of different studies;

- Examine multiple equation types and report information on all equation types considered, not just the equation providing best fit, to allow for better comparison of the results of different studies; and

- Address the questions:

  o What additional factors, other than temperature and moisture, might be required to accurately predict soil respiration?

  o Do study design and methods affect results or conclusions about the equation that describes temperature and moisture effects?

o What are the potential effects of respiration measurement technique,

incubation time, temperature range and/or moisture range?

Pseudoreplication, the use of statistical tests without treatment replication or with

replicates that are not statistically independent, should be avoided where possible and

acknowledged when present. Although pseudoreplication is present in many ecological

studies, unacknowledged pseudoreplication can result in improper conclusions and

interpretations of data due to inappropriate error terms (based on incorrect n) in the

statistical tests on which the conclusions are based (Hurlbert 1984). This study, as is

common with soil respiration incubation studies, includes pseudoreplication because of

limits of time, funding, and manpower. This study does not include replication of

temperature treatments, as four incubators each set to a different temperature were used

– a common component of the experimental design of studies examining temperature

controls of soil respiration. Due to the lack of true replication of the temperature treatment,

all moisture treatments for each temperature were grouped within one incubator (*i.e.,* the

moisture treatments were not independent), which led to inability to directly assess the

temperature-moisture interaction by using multiple regression or 3-factor ANOVA (factors =

layer, temperature, moisture). The following data analysis strategies were used in this study

to avoid improper interpretation:

- In the high moisture study, each incubation dish was quantified by a time-

  weighted respiration rate. Otherwise the multiple measurements over time

  would have been pseudoreplicates.

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- In the low moisture study, each incubation dish was quantified by a single respiration value, which was derived from multiple measurements over time. Otherwise, the multiple measurements would have been pseudoreplicates.

- Statistical analysis was performed among moisture treatments within each incubation chamber (Figure 2). Otherwise, moisture and temperature treatments would have been improperly treated as independent.

- In the statistical analysis to determine whether the moisture effect (respiration–moisture slope) varied with temperature, regression analysis used an average respiration value at each of the n = 4 temperatures. Otherwise, the multiple measurements per temperature would have been pseudoreplicates.

- Statistical derivation of Q_{10} used an average respiration value for each of the n = 4 temperatures. Otherwise, the multiple measurements per temperature would have been pseudoreplicates.

In calculating the equation to describe the temperature-moisture effect, however, multiple moistures at multiple temperatures were needed to determine an equation to describe the overall response of soil respiration. Pseudoreplication occurred due to the lack of multiple incubation chambers per temperature. To minimize the effects of pseudoreplication, summarized respiration rates for four moistures per temperature were used to determine the equations. This is not a challenge unique to the present study; due to the limitation of space, materials, time and funding, many similar soil respiration studies

have presented results that were obtained from data that may not have been independent (*e.g.,* moisture treatments within one incubator) and did not include true temperature replication (*e.g.,* only one incubator per temperature). Ideally, studies should use a fully factorial design with true replication of temperature treatments and a wide range of moistures to avoid pseudoreplication.

Future studies should examine several different equations to describe temperature and moisture controls of respiration, and report all significant equations, not just the equation with the best fit. It is possible that a common equation can be found that adequately predicts respiration for a variety of different soils and ecosystems, even if it is not the best equation for any particular study site. Each of the organic soil incubation studies compared here reported different equations that best described the relationship of temperature and moisture and their effect on respiration. However, as these authors did not report the forms of other equations examined, it is unclear which equation forms were considered in each study, and if the equation forms examined varied from study to study. The different best fit equations may be explained by researchers examining only a few equation forms, with different researchers examining different equations with little or no overlap from study-to-study. Alternatively, the nature of the temperature and moisture effects, and their interaction, may be site specific. In the future, researchers should try a variety of equation types, report which were examined, and provide information on all equations, not just the best fit. Researchers should keep in mind that different equations are needed to represent different moisture classes (*e.g.,* low moisture, high moisture or

very high [saturated] moisture). If any one moisture class is considered alone, such as low

moistures considered by Van Meeteren et al. (2007), Leiros et al. (1999), and the low

moisture drying study portion of this research, simple linear temperature or moisture terms

may be sufficient to describe the respiration response. When multiple moisture classes are

represented by the same equation, quadratic terms, logistic equations and other more

complex equation types(*e.g.,* such as those described by Bowden et al. 1998 and O'Connell

1990) may be needed to describe the response of respiration. If a common equation can be

found to sufficiently predict respiration from temperature and moisture for multiple soil

types and soil layers, future studies should examine the factors that explain differences in

coefficients from one site or layer to another.

4.10   Conclusion

This study found that, for the upper O-layer at low moistures (<230%), temperature

and moisture interact in their effect on soil respiration. This temperature–moisture

interaction was best described in statistical models as a complex interaction, in which the

proportional response of respiration to increased temperature increased with greater

moisture. At high moistures (200–500%) for both upper and lower O-layers, temperature

played the predominant role in influencing soil respiration, with moisture having little or no

effect. The upper O-layer had a higher respiration rate than the lower O-layer at all

temperature–moisture treatment levels, but the respiration response of the two layers to

temperature and moisture did not differ. Net N immobilization was not significantly

influenced by either temperature or moisture, but more N was immobilized in the upper O-layer than the lower O-layer.

Due to lack of consensus in the literature on the nature of the moisture effect, future laboratory incubation studies should ensure that treatments provide a range of moistures that include sufficiently high and low moistures that are likely to result in measurable respiration limitation. Inclusion of very high and very low moistures will allow researchers to refine the shape of the respiration response curve for a variety of soils.

Similarly, lack of consensus in the literature of the nature of the temperature–moisture interaction, or the existence of one in all soils, requires resolution. Studies need to be designed so that the temperature–moisture interaction can be analyzed with high statistical validity. Understanding which equation(s) best describe the effects of temperature and moisture on soil respiration is important for predicting long-term changes in global C budgets. While the CENTURY and Rothamsted models assume a multiplicative effect of temperature and moisture, this and other research has shown a more complex temperature–moisture interaction. Ultimately, this complex interaction may need to be taken into account in global C budget models to better predict changing C fluxes as a result of global climate change.

Due to the very low number of studies examining multiple organic soil or litter layers, or comparing mineral and organic soil respiration, additional studies of these comparisons are needed to determine whether soil layers display similar or different

temperature and moisture effects. The results of this study, which found no difference in

moisture or temperature effect between the two O-layers, support the CENTURY and

Rothamsted models' assumption that all soil layers respond similarly to temperature and

moisture, but are in conflict with the results of other studies that have found different

temperature effects (Q_{10}) for different soil layers and soil depths. Other studies indicate

that there are differences in temperature and moisture effects on soil respiration in soils

with different substrate quality or between organic and mineral soils. Additional research is

needed to determine the exact nature of these differences, and further exploration is

needed to explain the cause of these differences, if they do indeed exist. Further, additional

studies are needed to adequately describe the extent and nature of the coupling of C and N

dynamics in soils under potential climate change scenarios. Ultimately, lack of consensus on

the nature of the temperature–moisture interaction in different soils and soil layers means

that additional studies are required to determine whether a single response function is

sufficient to describe this effect for all soils, or if the response of soil respiration is site

specific and requires additional predictive terms.

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6. Appendix

A1. Incubation Temperatures

Soil incubation temperature was monitored throughout the incubation using two Thermochron iButtons per chamber (Table A1).[3] Average measured temperature was within 1°C of nominal incubation temperature.

Table A1. Nominal and measured incubation temperatures (n = 2).

Nominal Incubation Temperature (°C)	Average Measured Temperature (°C)	Standard Deviation	±95% CI
5	5.8	0.17	1.5
10	9.7	0.14	1.3
15	14.8	0.10	0.93
20	19.4	0.17	1.5

A2. Effect of Time on Respiration

Concerns regarding the effects of incubation time arise in all but very short-term incubation studies. In this study, an additional concern is the confounding of time and moisture in the low moisture drying study (study 2). Time effect was examined for both the high moisture and the low moisture drying studies.

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[3] Average measured temperature was used for all statistical analyses and in figures. However, for ease of reference, nominal temperature was used when temperature chambers were not directly compared, in tables and when discussing the incubation temperatures in the text.

A2.1   Effect of Time on Respiration at High Moisture

What is the temporal pattern of respiration?

Figure A1 and Figure A2 below illustrate two examples of the temporal response of respiration throughout the high moisture study. Figure A1 illustrates a dish with a significant decrease in respiration with time. Figure A2 illustrates a dish with a non-significant response of respiration over time.

The equation shown on the plot is:

$$y = 0.005x^2 - 0.239x + 6.294$$
$$\text{Adj. } R^2 = 0.893,\ p < 0.001$$
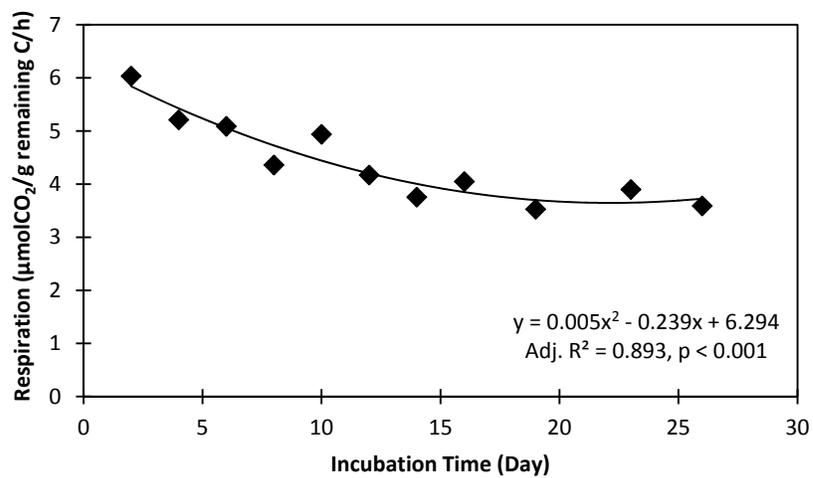
Figure A1. Respiration rate by incubation time for lower layer soil at 300% moisture incubated at 20°C (Dish 45). Each diamond represents the average of three respiration measurements for one day for this dish.
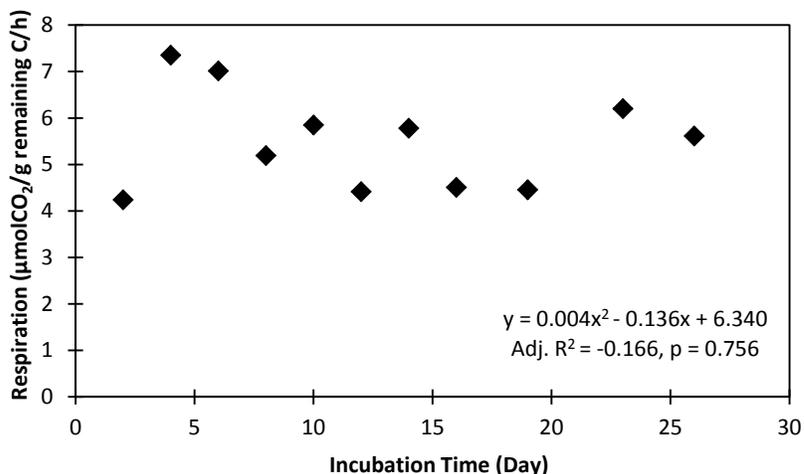
Figure A2. Respiration rate by incubation time for upper layer soil at 500% moisture incubated at 10°C (Dish 30). Each diamond represents the average of three respiration measurements for one day for this dish.

Linear and quadratic regression analysis was performed on each dish individually to determine if average respiration rate (μmolCO$_2$/g remaining C/h) was dependent on incubation time. For the upper layer, 6 out of 32 dishes (19%) had significant regressions (Table A2). For the lower layer, 17 out of 32 dishes (53%) had significant regressions. Change in respiration rate from day 1 to day 27 was determined for each dish from its regression equation. Average change in respiration rate differed from zero for five of the eight temperature-layer combinations ($p < 0.05$, Table A2). Average respiration rate declined by as much as 35% from day 1 to day 27 (lower layer, 10°C).

Table A2. Temporal change in respiration at high moisture, as indicated by linear and quadratic regression (respiration vs. time), and change in respiration from day 1 to day 27.

Layer	Temp (°C)	Number of Dishes with Significant Regression (out of 8 dishes)	Change in Respiration from Day 1 to 27 ($\mu mol CO_2$/g remaining C/h)		T-Test Result (H_0: change=0) p-value (n=8 dishes)
			Average	Standard Deviation	
Upper	5	1	-0.8	1.4	0.144
Upper	10	1	-0.7	0.7	0.038
Upper	15	0	-0.2	1.4	0.703
Upper	20	4	-2.7	1.5	0.001
Lower	5	3	-0.3	0.6	0.158
Lower	10	4	-1.0	0.6	0.001
Lower	15	4	-0.8	0.6	0.004
Lower	20	6	-1.6	0.9	0.001

A2.2   Effect of Time on Respiration at Low Moisture

What is the temporal pattern of respiration?

During the low moisture drying study, respiration tended to decrease as moisture decreased and incubation time increased (e.g., Figure A3).
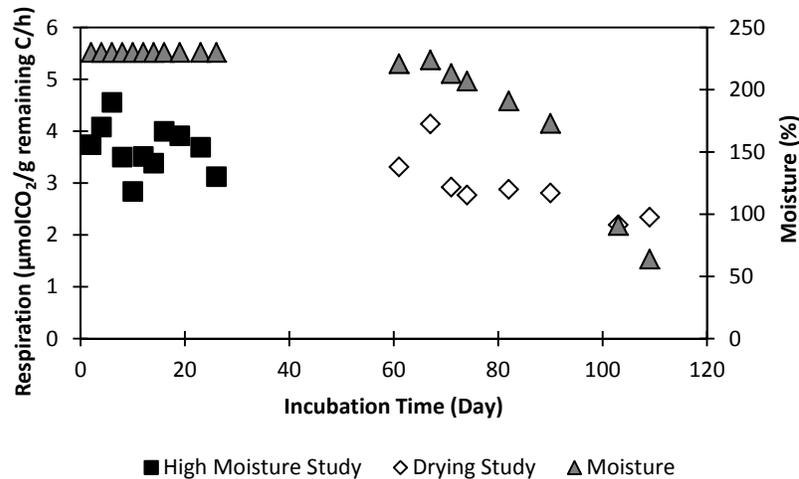
Figure A3. Respiration and moisture by incubation time for both studies for upper layer soil at 5°C (Dish 33). Each square and diamond represents the average of two respiration measurements for one day for this dish. Each triangle represents moisture measurement for one day taken immediately prior to respiration measurement.

The decline in respiration rate could be due to either increased incubation time or decreased moisture. In the 35 days prior to commencement of the drying study, some decline in respiration occurred at constant high moisture in three of five layer–temperature combinations (Table A3); whether this decline with time continued during drying is not clear. Dishes at the same temperature and different initial moistures reached 160% moisture at different times, but had similar respiration rates at 160% moisture (Figure A4), suggesting time alone was not influencing the decrease in respiration rate. Therefore, the

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decline in respiration associated with the low moisture drying study is more likely attributed

to decreased moisture rather than increased incubation time.

Table A3. Change in respiration at constant high moisture prior to drying study, from incubation day 27 (last day of high moisture study) to day 61 (first day of low moisture drying study).

| Layer | Temp (°C) | Respiration (μmolCO$_2$/g remaining C/h) Average of 8 Dishes | | | |
		Average Respiration on Day 27	Average Respiration on Day 61	Change from Day 27 to 61	Paired T-Test Result (p-value)
Upper	5	3.8	3.5	-0.3	0.312
Upper	10	4.8	4.3	-0.5	0.068
Upper	15	6.7	5.8	-1.0	0.001
Upper	20	10.7	9.9	-0.8	0.031
Lower	20	4.0	3.8	-0.2	0.372
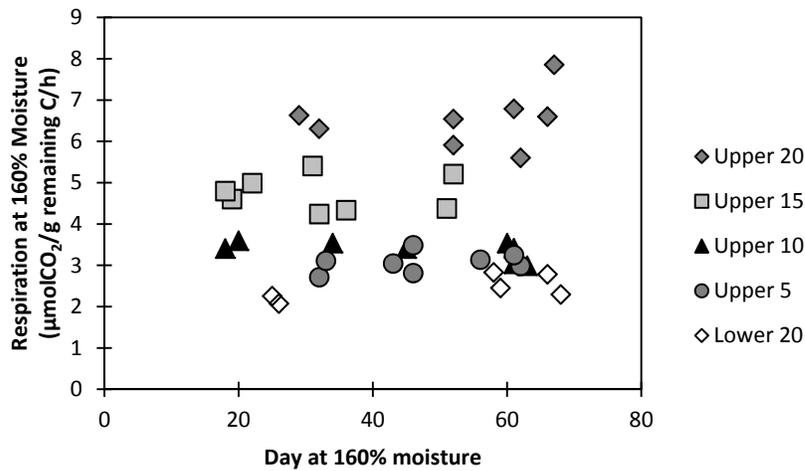
Figure A4. Respiration at 160% moisture by incubation time for each layer-temperature combination. Each symbol represents the calculated respiration rate at 160% moisture for one dish.

A3. Respiration

A3.1 Study 1: Effect of Layer, Temperature and High Moisture on Respiration

A two-factor ANOVA (four moistures and two layers fixed) found that neither layer–moisture interaction nor moisture had a significant effect on respiration at any temperature (Table A4). Figure A5 illustrates time-weighted respiration rate by moisture at 20°C as an example.

Table A4. *P*-values for two-factor ANOVA (fixed factors = 2 layers, 4 moistures) at high moisture.

Temp (°C)	n*	Layer-Moisture Interaction p	Moisture p	Layer p	For Pooled Moisture, Amount Upper Layer Respiration Rate is Greater Relative to Lower Layer Rate (%)
5	2	0.24	0.55	<0.001	92%
10	2	0.51	0.25	<0.001	123%
15	2	0.83	0.34	<0.001	148%
20	2	0.46	0.25	<0.001	135%

*n = number of dishes for each of moisture-layer combination (four moistures and two layers)
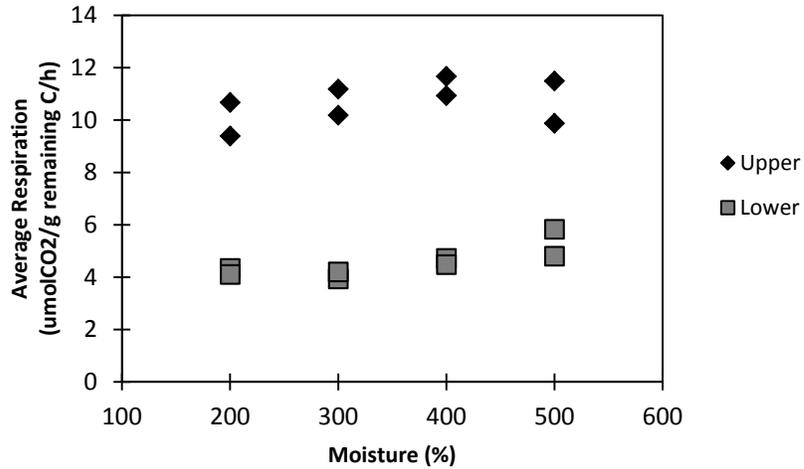
Figure A5. Time-weighted average respiration by moisture and layer for 20°C samples at high moisture. Each diamond and square represents time-weighted average respiration rate for one dish at 20°C (two replicate dishes per moisture for each layer).

As moisture was not found to significantly affect soil respiration for any temperature chamber (see previous section), moisture was pooled for each temperature chamber and layer combination.

Linear regression analysis was conducted to determine if incubation temperature had an effect on ln-respiration. Average pooled respiration for the upper layer increased by 180% from 5°C to 20°C (Figure A6**)**. Average pooled respiration for the lower layer increased by 129% from 5°C to 20°C. The upper layer had a respiration rate that was on average 2.2 times higher than the respiration rate for the lower layer.

$$\ln(\text{Resp}) = 0.886 + 0.076 \times T$$
Adj. $R^2 = 0.977$, $p < 0.001$

$$\ln(\text{Resp}) = 0.261 + 0.061 \times T$$
Adj. $R^2 = 0.820$, $p < 0.001$

■ Upper Layer
◆ Lower Layer
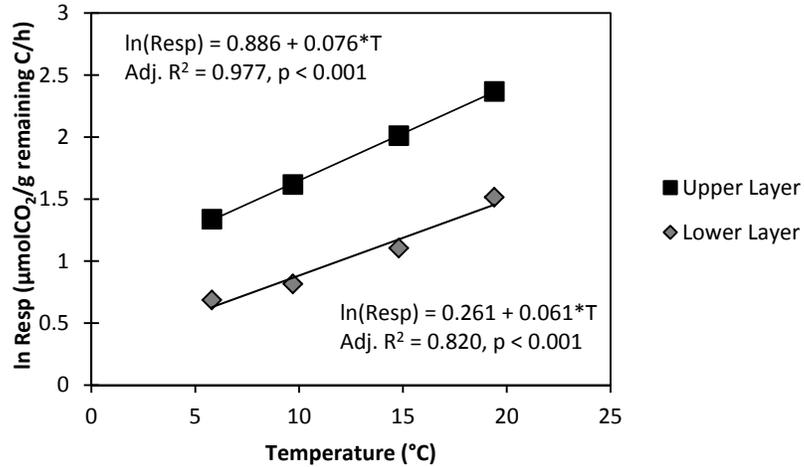
Figure A6. Average respiration by measured incubation temperature for upper and lower layers. Each diamond represents the average respiration rate for eight dishes per temperature-layer combination (200-500% moisture).

A3.2   Study 2: Effect of Layer, Temperature and Low Moisture (<230%) on Respiration

Linear regression analysis of the effect of moisture on respiration was performed on each dish for moistures below 230%. Average slope (change in respiration/change in moisture) was calculated for each temperature for the upper layer, and for 20°C for the lower layer. For each layer–temperature combination, a one-sample t-test found that the average slope differed from zero (Table A5).

Table A5. Summary of slope (change in respiration [μmolCO$_2$/g remaining C/h]/change in moisture [%]) from linear regression analysis for moistures below 230%.

Layer	Temp (°C)	n* (dishes)	Average Slope	±95% CI	T-Test Results (H$_0$: slope=0) p-value
Upper	5	7	0.0043	0.001551	< 0.001
Upper	10	7	0.0093	0.003144	< 0.001
Upper	15	6	0.015	0.004077	0.001
Upper	20	6	0.032	0.006469	0.002
Lower	20	5	0.023	0.016508	0.012

*There are a different number of dishes for each layer-temperature combination because not all dishes had more than three respiration measurements at moistures at or below 230%. Minimum of three respiration measurements are required for linear regression analysis.

A3.3   Synthesis of Studies: Effect of Layer, Temperature and Moisture on Respiration at Low and High Moistures

Is there an interactive effect of temperature and moisture on respiration?
Are the two layers similar or different?

The Q_{10} for the upper layer increased with moisture at low moistures (<230%) but

did not increase above 230% moisture, indicating that the effect of temperature on

respiration depends on moisture (Table A6). Average Q_{10} at high moisture was 2.0.

Table A6. Q_{10} by layer and moisture

Layer	Moisture	Q_{10}	Range (min-max)
Upper	100	1.3	1.13-1.42
Upper	140	1.6	1.04-1.42
Upper	180	1.8	1.44-2.88
Upper	220	1.9	1.52-2.97
Upper	230	2.1	1.75-2.61
Upper	300	2.1	1.71-2.55
Upper	400	2.1	1.71-2.55
Upper	500	2.2	2.03-2.47
Lower	200	1.6	0.59-4.27
Lower	300	1.8	1.61-1.96
Lower	400	1.8	1.20-4.25
Lower	500	2.3	1.29-4.25

Is the response of O-horizon respiration to temperature and moisture described by a unique statistical model?

Several equations were fitted to upper layer data from the low moisture drying study. To properly compare these equations using Akaike Information Criterion (AIC) analysis, all equations are for ln(Respiration). Equations for respiration in non-transformed units are converted to ln(Respiration) (*e.g.,* Resp = a + bT + dTM converted to ln[Resp] = ln[a + bT + dTM]). A full review of the equations examined is listed in Table A7.

Table A7. Summary of linear regression results for upper layer, low moisture data.

Equation Type	Intercept a (SE)	Temp b (SE)	Moisture c (SE)	Temp x Moisture d (SE)	Temp^2 e (SE)	Moisture ^2 f (SE)	Stats for the Equation/Estimate Adj. R^2	p	SE for estimate	AIC Analysis Delta AIC(c)	AIC Rank
Additive effects											
ln(Resp) = ln(a + bT + cM)	0.27	0.20	1.05				0.814	N/A	N/A	28.2	13
Multiplicative effects											
ln(Resp) = ln(dTM)				0.25			0.050	N/A	N/A	13.9	6
ln(Resp) = dTM				0.064			-0.93	N/A	N/A	62.3	17
ln(Resp) = a + bT + cM	0.33** (0.10)	0.050** (0.0043)	0.31** (0.049)				0.922	<0.001	0.09	15.8	7
ln(Resp) = a + bT + cM + eT^2 + fM^2	0.29 (0.39)	0.037 (0.030)	0.45 (0.47)		0.00051 (0.0012)	-0.043 (0.15)	0.910	<0.001	0.09	23.4	11
ln(Resp) = ln(20.0 * [0.13*e^(0.057T)] * [0.33 + 0.0031M]) (CENTURY-type equation)							0.83	N/A	N/A	33.4	14

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Table A7. Summary of linear regression results for upper layer, low moisture data. (Continued)

Equation Type	Intercept a (SE)	Temp b (SE)	Moisture c (SE)	Temp x Moisture d (SE)	Temp^2 e (SE)	Moisture ^2 f (SE)	Adj. R^2	p	SE for estimate	Delta AIC(c)	AIC Rank
Complex effects											
ln(Resp) = ln(a + dTM)	1.91			0.13			0.91	N/A	N/A	13.9	6
ln(Resp) = ln(a + bT + cM + dTM)	2.75	-0.05	-0.60	0.17			0.92	N/A	N/A	17.8	9
ln(Resp) = ln(a + cM + dTM)	2.05		-0.34	0.15			0.90	N/A	N/A	17.4	8
ln(Resp) = a + cM + dTM	0.95** (0.050)		-0.074 (0.036)	0.031** (0.0016)			0.97	<0.001	0.054	6.0	4
ln(Resp) = ln(a + bT + dTM)	1.58	0.21		0.13			0.89	N/A	N/A	19.1	10
ln(Resp) = a + bT + cM + dTM	0.87** (0.13)	0.0066 (0.010)	-0.026 (0.081)	0.027** (0.0060)			0.969	<0.001	0.06	3.5	3
ln(Resp) = a + bT + dTM	0.83** (0.035)	0.0095* (0.0044)		0.026** (0.0022)			0.971	<0.001	0.05	0	1

Table A7. Summary of linear regression results for upper layer, low moisture data. (Continued)

Equation Type	Intercept a (SE)	Temp b (SE)	Moisture c (SE)	Temp x Moisture d (SE)	Temp^2 e (SE)	Moisture ^2 f (SE)	Adj. R^2	p	SE for estimate	Delta AIC(c)	AIC Rank
Complex effects (Continued)											
$\ln(Resp) = a + dTM$	0.87** (0.033)			0.029** (0.0015)			0.964	<0.001	0.06	1.8	2
$\ln(Resp) = \ln(a + bT + cM + eT^2 + fM^2)$	-0.057	0.017	1.97		0.086	-0.13	0.69	N/A	N/A	45	15
$\ln(Resp) = \ln(a + bT + cM + dTM + eT^2+fM^2)$	3.84	-0.30	-0.46	0.20	0.0086	-0.13	0.92	N/A	N/A	24.1	12
$\ln(Resp) = a + bT + cM + dTM + eT^2 + fM^2$	0.84** (0.28)	-0.0062 (0.021)	0.11 (0.31)	0.027** (0.0064)	0.00051 (7.4E-4)	-0.043 (0.092)	0.965	<0.001	0.06	12.1	5

*P< 0.001** P< 0.1*

N/A = not available; converted equations were solved using Excel Solver, therefore, p-value and standard error for coefficients or the estimate were not available.

A4.   Net N Mineralization

What is the relation of net N mineralization to temperature and moisture?

A two-factor ANOVA (four moistures and two layer fixed) for net N mineralization was conducted for each temperature after 27 days of incubation. Neither layer–moisture interaction nor moisture had a significant effect on the ammonium concentration at any temperature (Table A8). The lack of moisture effect at each temperature indicates there is no interactive effect of temperature and moisture on ammonium concentration at high moistures. The upper layer had a significantly greater negative net N mineralization rate than the lower layer at all temperatures. Since negative N mineralization rate denotes net N immobilization, this means that the upper layer had a higher rate of net N immobilization than the lower layer.

As moisture was not found to significantly affect net N mineralization at any temperature, moisture was pooled for each temperature and layer combination. Regression analysis did not find a significant temperature effect on net N mineralization for either layer (Table A8).

Table A8. Post-incubation results for net N mineralization. Two-factor ANOVA (fixed factors = layer, moisture) and regression (temperature vs. net N mineralization) results at high moisture. Note that negative mineralization is immobilization

		ANOVA Results			
Temp (°C)	n (dishes per moisture-layer combination)	Layer-Moisture Interaction p	Moisture p	Layer p	Amount Upper Layer Net N Immobilization is Greater Relative to Lower Layer (%)
5	2	0.261	0.473	0.003	61
10	2	0.176	0.148	0.002	75

15	2	0.241	0.280	<0.001	62
20	2	0.144	0.191	<0.001	65

Table A8. Post incubation results for net N mineralization. Two-factor ANOVA (fixed factors = layer, moisture) and regression (temperature vs. net N mineralization) results at high moisture. Note that negative mineralization is immobilization. Continued

Regression Results					
Layer	n (average for each of 4 temperatures)	Average Net N Mineralization (μg NH$_3$-N/g dry soil)	95% CI	*P*	Adj. R^2
Upper	4	-7.04	2.6	0.255	0.332
Lower	4	-2.5	1.4	0.426	-0.005

A5.   Summary of Temperature and Moisture Effects from Other Studies

A review of the literature revealed that various equation types for temperature and moisture effects have been found by studies examining the respiration response to these variables in laboratory incubations of organic soils, laboratory incubations of mineral soils, and field studies (Table A9).

Table A9. Summary of temperature and moisture effects on respiration and equation type for other studies.

Type of Temperature and Moisture Effects	Equation type	Reference
No significant moisture effect	N/A	Howard and Howard 1979, Mathes and Schriefer 1985, Edwards 1975, Waldrop and Firestone 2004, Fang and Moncrieff 2001
Temperature (+), Moisture (+) relationship with Respiration	No equation provided	Guntinas et al. 2013
Temperature (+), Moisture (-) relationship with Respiration	No equation provided	Shaver et al. 2006
Temperature-Moisture interaction confirmed	No equation provided	Klimek and Niklinska 2010
Additive effect	$Resp = T + M$	Chang et al. 2012, Van Meeteren et al. 2007
Complex effect	$Resp = T + M + TM$	Leiros et al. 1999, Raich and Schlesinger 1992
Complex effect	$\ln(Resp) = T^2 + T + M^2 + M + TM$	Bowden et al. 1998
Multiplicative effect	$Resp = (e^T)*(e^M)$	Davidson et al. 1998
Multiplicative effect	$Resp = (e^{1/T})*(A^M)$	DeForest et al. 2009
Multiplicative effect	$Resp = (\exp (A + B*(1 - C*T/B))) / (1 + D*\exp(E*M))$	O'Connell 1990
Multiplicative effect	$\ln(Resp) = T^2 + T + M^2 + M$	Rey et al. 2005, Howard and Howard 1993
Temperature and Moisture effects analyzed separately	$Resp = T^2 + T$; $Resp = M$	Azzalini and Diggle 1994
Temperature and Moisture effects analyzed separately	$\ln(Resp) = T$; $\ln(Resp) = M^2 + M$	Howard and Howard 1993

Note: A, B, C, D, and E are constants; T = Temperature; M = Moisture; Resp = soil respiration