Study Plan
Spatial and temporal variation in soil respiration as a basis for ecosystem analysis

Pacific Northwest Research Station
Ecosystem Processes Program

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Background

Context

The efflux of CO$_2$ from the soil surface (here called soil respiration or Rs) is one of the largest fluxes of the global C cycle, larger than NPP and second only to GPP (Raich and Schlesinger 1992; Rustad et. al. 2000; fig. 1). For this reason and others, ecologists have attempted to estimate Rs in many ecosystems (e.g., Raich and Potter 1995, Raich and Schlesinger 1992). Rates of Rs have also interested, among others, ecologists studying ecosystem function, energy flow, and especially belowground ecology.

![Diagram of the C cycle](image)

Figure 1. The global C cycle (pool units are 10$^{15}$ g C; fluxes are 10$^{15}$ g C y$^{-1}$) averaged for the 1980s. Modified from Schlesinger (1997).

Methodological limitations

Methods to measure Rs to date, however, are largely unproven and are difficult to apply to field conditions. They are unproven in the sense that methods vary widely and are rarely checked with known control fluxes, samples are poorly distributed across space and time, and more rigorous statistical techniques are rarely used. Most previous work has focused on obtaining estimates, usually by sampling along a transect, and sometimes repeating sampling during the growing season—thus, average rates can be presented with
Confidence intervals. Consider, however, that chambers rarely exceed 20-cm in diameter (0.000004 ha), so even 100 samples covers 4/100ths of a ha. Measured in about 1 minute, IRGA-based rates also represent only 1/525,600th of a year. With such a low sampling intensities, we wonder if rates and intervals, although they may look reasonable, are far from accurate or repeatable. Further, sampling at fixed intervals along a transect may produce misleading estimates if spatial autocorrelation is present, and few studies include enough sampling points to adequately assess this. Spatial autocorrelation has been found in agricultural fields at scales of less than 0.5 m (Nakayama 1990 and Rochette et al. 1991) and is likely in forests as well. We, and others, have demonstrated that easy-to-use chemical absorption methods (for example, soda-lime chambers) have large concentration-gradient-driven biases (Nay et al. 1994, Heally et al. 1996, and Jensen et al. 1996); gas-flux methods using infra-red gas analyzers (IRGA) do work under laboratory conditions but are limited to short sampling periods and are time consuming in the field. For example, Rochette et al. (1991), working in an agricultural field using a dynamic-chamber-IRGA method (much like our methodology), found that between 33 and 190 sample points were needed to be within 10 percent of the mean, with 95 percent confidence. Current IRGA techniques require about 5 min per sample, thus to achieve this precision would take 3 to 16 h.

Inherent variation

Seasonal differences have been observed by a number of investigators (Joshi et al. 1991, Garrett et al. 1978, Rochette et al. 1991, Gordon et al. 1987) with highest rates occurring during the summer months, perhaps coinciding with changes in moisture, temperature or photosynthate production. In the Joshi et al. (1991) study in the Central Himalayas, high rates corresponded with the rainy season. Kurser (1989) found soil profile concentrations of forests in Panama to nearly double during the two-month rainy season. Jensen et al. (1996) suggests using a model with precipitation events to better account for some of the day-to-day fluctuations in Rs. A small amount of precipitation with a small effect on soil moisture can have a large effect on gas diffusion. Information on diurnal variation in Rs is less extensive. In a grassland study, Grahammer et al. (1991) generally found higher rates during the day, which were attributed to photosynthate production. On the other hand, Garrett et al. (1978) found little diurnal change in Rs in an Oak-Hickory forest, despite their observance of significant diurnal differences of ambient CO₂ concentrations in the air above the soil surface.

Most field data show high temporal and spatial variability (e.g., Rochette et al. 1991 and Jensen et al. 1996). We have collected similar data sets in a wide range of conditions in the Pacific Northwest and southeast and interior Alaska (fig. 2). Even the most ideal situations such as agricultural fields or even well-controlled mesocosm experiments show high variation. Griffins et al. (1996) found spatial variation to be high in a mesocosm experiment where soil respiration rates ranged from 4 to 25 μmol m⁻² s⁻¹ CO₂ for 150 sample points measured over a two day period on an area of 3.6 m². Nay and Bormann
(2000) found similar variability in a box-lysimeter experiment with homogenized soils and no plants (fig. 3) where significant differences ($\alpha=0.05$) were detected at 25-cm intervals within 24 h.

**Figure 2.** Variation in Rs over a growing season along a 20-m transect in a mature Doug-fir forest near Scio, OR. Multiple points represent diurnal variation. Note the persistence of some “hot spots” and changes in diurnal variation. Unpublished data courtesy of Mark Nay and Kim Mattson.

**Figure 3.** Spatial variation in box-lysimeter experiment with initially homogenized soil and no plants (Nay 1994).

**Understanding sources of variation better**

We have learned from more than a decade of Rs measures that the high variation in Rs, typically seen in field studies, likely comes from many sources, some which are not easily foreseen. Additional sources we know little about now are likely to emerge as well from a more systematic study. Gas-flux methods have been a large source of variation and bias, for example, using soda-lime chambers that introduce large concentration-gradient-driven biases and chambers without soil collars that force CO$_2$ into the chamber when pressed on the litter layer. Other known sources include:
- Soil moisture (Singh and Gupta 1977; Howard and Howard 1979; Shclenter and Van Cleve 1985; Davidson et al. 1998, 2000), 
- Precipitation (Simunek and Suarez 1993 and Jensen et al. 2000), 
- Substrate quality (Tewary et al. 1982), (5) vegetation (Raich and Schlesinger 1992, and Raich and Tufekcioglu 2000), and 

Soils are likely more complex than often assumed—so other sources of variation should be anticipated. Soils are affected by a complex history of disturbance, biological effects from roots, tunneling soil animals, fungi, and microbes engaged in many processes such as soil mixing, weathering, and decomposition. Trees alter rain infiltration and amount of soil water, and deposit litter heterogeneously. Different plants affect soils and microbes in ways that are likely to alter Rs as well. We have seen glimpses of previously unrecognized physical processes such as chimney and barrier effects. Even in homogenized soil, we have seen long-lasting hotspots that are probably best explained by columns of high-diffusivity soil, intense biological activity, or adjacent low diffusivity soil barriers. Studies of soil variation appear to parallel, and may relate to Rs variability. In windthrow-affected soils of southeast Alaska, for example we see large variation in O horizon thickness and composition and in the extent and thickness of podzolic E and Bh horizons, that may hinder gas flow (fig. 4). Taken together, we conclude that a more systematic approach is needed to better understand known, and identify new, sources of variation as a basis for determining if reasonable Rs measures can be obtained in field studies, and concurrently to modify methods to better deal with this variation.

Figure 4. Variation in O, E, and Bh horizon thickness along 1-m transects on poorly productive till and highly productive colluvium soils on Chichagof Island in southeast Alaska. The O and Bh horizons are C rich, but the Bh horizon become dense, can create a perched water table, and likely hinders gas flux.
Justification

Improved methods to estimate soil Rs, based on a better understanding of the magnitude and patterns of known and potential variation, will likely be useful to studies of the global C budget, ecosystem energy flows, and belowground ecology. The initial justification for this study is to see if we can feasibly measure Rs in 2-ha field plots as part of the PNW Station Long-Term Ecosystem Productivity (LTEM) program. The LTEM approach to measuring net primary productivity modifies the approach of Grier et al. (1989):

\[
\text{Npp} = \Delta B + D + G,
\]

where \(\Delta B\) is 5-yr change in living biomass, \(D\) is detritus production, and \(G\) is consumption via herbivory. The LTEM approach focuses on long-term measures of \(D\) plus \(G\) as: change in soil organic matter (\(\Delta D\)) plus heterotrophic respiration (\(Rh\), estimated as average annual Rs minus an estimated belowground autotrophic respiration), minus annual inputs minus outputs of biomass and detritus from the defined ecosystem (I-O):

\[
\text{Npp} = \Delta B + \Delta D + Rh - (I-O).
\]

This formulation fully depends on estimates of Rs that have a precision reasonably similar to \(\Delta B\) and \(\Delta D\), which are fairly well known for our field sites (Homann et al. 2001). Even with precise measures of Rs, however, this method will require estimating the proportion of Rs that is Rh, a problem no one has satisfactorily solved to date, however work there is a growing body of work on this topic (Hanson et al. 2000). The benefit of this approach is that estimated belowground components of productivity are bounded by actual measures of Rs, unlike the alternative, root-turnover methods (Vogt et al 1986, Lauenroth et al. 1986). Measures of Rs in the field could be applied to many other problems as well, including a direct assessment of combined biological activity of heterotrophs and autotrophs.

Study Objectives

The purposes of this study are to consider a wide array of potential sources of spatial and temporal variation, explore patterns of variation at multiple scales of time and space, identify dominant sources of variation in different forest types through systematic sampling at multiple scales, and then to develop more efficient ways to measure stand- and annual-scale Rs with a precision fitted to the analysis being used. Specific objectives are:

1. Compare Rs rates and patterns of variation in 4 spatial and temporal scales in 2 to 3 forested sites;
2. Examine soil under sample points and evaluate stand characteristics that may relate to Rs variation or that could be used as surrogate measures or amplifying covariates; and
3. Modify current techniques to maximize precision of field measures.
Details for objective 1 (rates and patterns)

Spatial scales. Three scales will be evaluated with 2-, 1-, and 0.5-, and 0.25-m nested hexagonal grids (fig. 5). Methods for measuring Rs are described under objective 3. Rochette et al. 1991 found spatial patterns at about 0.15 m for agricultural soils, thus working at spatial scales from 0.25 m to 2 m seems an appropriate starting point, given that forest soils are much more variable. Converting from transects to hexagons roughly doubles the number of comparisons that can be used to determine spatial autocorrelation. We expect only a minor decline in variability moving to the smallest scales, given limited experience so far. In general, we expect temporal variation to be far smaller than spatial variation. Quantifying these differences will have a large effect on design of an optimal sampling strategy. Once initial results are analyzed, even broader scales can be added to this design, such as 4, 8, and 16 m hexagonal grids, to insure that large-scale patterns are described as well.

Figure 5. Nested hexagonal sampling scheme.

Temporal scales. Temporal scales are yet to be established and will depend on completing a remote robotic sampling device (see objective 3 details). An initial idea is to measure all points as quickly as possible, again at 2x the time of one cycle, again at 4 and 8x the cycle. Additional programs can be applied later once the first tests are completed.

Sites. We will apply the nested hexagon grids to three different sites. We will apply one grid system to an LTEP plot with a 70-90 year-old forest at Hebo on the Sisulaw or Isolation Block on the Willamette National Forests. Depending on additional collaboration and funding, we would like to add an old-growth plot in the Pacific Northwest, and a southeast Alaska forest site, perhaps one of our soil variability plots on the Juneau road system. We expect variance in Rs rates to increase in this sequence: LTEP, old-growth, and wind-affected Alaska podzols, in concert with perceived soil variability. Stand-average rates of Rs would likely follow variance, but whether patterns will change is unknown.
**Statistical analysis.** Our strategy includes data visualization, analysis of spatial and temporal autocorrelation, and using traditional parametric statistics (regression and ANOVA) as appropriate. As an example, we will likely use a nested analysis of variance to assess differences in variances between estimates at different scales (table 1.). A similar design will be used to assess different temporal scales. SPLUS will be used for data visualization and to analyze patterns and determine autocorrelations. Standard techniques will be used to calculate the optimal spacing of sampling points in time and space, and the number of samples required to achieve different degrees of precision.

**Table 1. ANOVA table for comparison of spatial scales**

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0-m scale</td>
<td>11</td>
</tr>
<tr>
<td>1.0-m scale</td>
<td>17</td>
</tr>
<tr>
<td>0.5-m scale</td>
<td>17</td>
</tr>
<tr>
<td>0.25-m scale</td>
<td>17</td>
</tr>
<tr>
<td>Total</td>
<td>62</td>
</tr>
</tbody>
</table>

**Details for objective 2 (soil factors)**

After sampling has been completed, a quantitative soil pit (fig. 6) will be used to measure, a wide array of soil characteristics under each Rs sampling point. The quantitative-pit method permits calculation of attributes on a per-unit-area and volume basis. We intend to measure all relevant physical and biological factors likely to relate to observed Rs. This list includes C mass and quality; horizon structure, density, and possibly diffusivity; root, microbial, and fungal biomass (especially presence of mats); particle-size distribution, total and available nutrients, and water-holding capacity. These measures are standardized and are detailed in the LTEP standard operating procedures guidelines (on file). Samples will be prepared and added to the LTEP long-term sample base for other possible analyses as well. Diffusivity can be measured with a lab apparatus we developed for Nay et al. (1994).

**Figure 6. Vertical sampling frame, fixed in place with pins, for the quantitative-pit method—allowing sampling of visible horizons, fixed sample area, depths, and volumes.**
Details for objective 3 (modify techniques)

Typically, IRGA Rs samples are collected along a transect at a fixed interval. Measures are made on collars placed into the soil at all sample points several days prior. The collars are of the same diameter (20 cm) as the soil respiration chamber and work as a conduit to the soil to avoid disturbing the soil or pressing out concentrations of CO₂ at the time of measurement. Once on the collar, the chamber circulates air between the chamber and IRGA, and monitors the change in CO₂ concentration to estimate Rs.

The current IRGA technology we use is a LI-Cor http://env.licor.com/ LI-6200 Portable Photosynthesis System adapted for soil respiration measurements (fig. 7). This instrument has been very reliable and has become a standard for field portable IRGA’s, however the system weighs approximately 15 kg and is somewhat cumbersome for fieldwork particularly in forestry work with rough terrain and brush. The LI-6200 technology is very adaptable though and can be reconfigured and programmed to work for a wide range of applications. Although the LI-6200 is our current state of technology it is technology that is well over ten years old. The LI-6200 is no longer available and has been replaced with the LI-6400 system, which is slightly more compact and weighs approximately 12 kg (fig. 8). The cost of a LI-6400 is significant though at around $22.5 K. LiCor also sells a soil respiration chamber the LI-6400-09 at around $1.5 K to be used in conjunction with the LI-6400—we are concerned about the small size of this chamber.

Figure 7. Li-Cor LI-6200 Portable Photosynthesis System and soil CO₂ flux chamber.
In this study we would like to explore several changes in how to make soil respiration measurements with IRGA technology to increase the number of samples we can collect in a day's time:

- Use hexagonal sampling patterns rather than transects (already discussed);
- Develop a lightweight IRGA soil respiration chamber system; and
- Employ robotics for moving and operating IRGA and soil respiration chamber to obtain diurnal and other time dependent measures without an operator present.

Any of these innovations, if adopted, should be able to increase sampling efficiency. We will assemble a lightweight IRGA soil respiration system with a new lightweight (1 kg) IRGA the LI-800 (Li-Cor Inc., Lincoln, NE) now available for approx $2.5 K (fig. 9). The LI-800 is only an IRGA and does not contain data logging capabilities, an airflow pump, or batteries. These additional components combined with a soil respiration chamber would make for a field apparatus that would weigh from 6 to 7 kg. The IRGA and soil respiration chamber combined into a single unit would make the instrument more ergonomic for field use. The system would be designed with only one protocol simplifying setup and insuring consistency in the field and among multiple users. This method can measure Rs and soil and air temperature simultaneously. We will explore the possibility of measuring soil moisture as well. Other modifications are likely to emerge after analyzing patterns of variation, for example chamber size might be modified to encompass more small-scale variation.

Figure 9. Li-Cor, LI-800 IRGA.
We will also explore the use of robotics to maneuver the IRGA and chamber from sample point to sample point on a timed program. Because of the point nature of sampling with the IRGA in both space and time, a large number of sequential measurements are needed. This type of work can be quite monotonous and tedious, more suited for a robot than a technician. Robotics is a much growing field—information and components are readily available via the internet by any number of University, government, private companies and robot clubs [http://www-robotics.cs.umass.edu/robotics.html, http://www.ri.cmu.edu/]. Microcontrollers such the Basic Stamp (Parallax Inc., Rocklin, California, http://www.parallaxinc.com/ ) are easily programmable, inexpensive with lots of source code readily available. We propose building a very simple robot that can position the IRGA and soil respiration chamber onto sampling collars (fig. 9). We have consulted with Gene Koriene, (Ph.D., Chief Scientist for 3 Sigma Robotics, Corvallis, OR; http://www.3sigmarobotics.com/home.html), who reviewed the conceptual design of this project and offered additional help.

Figure 9. A possible robotic system with an actuator arm that can visit sampling points and collect data robotically on a specified program.

Timeline

Develop lightweight IRGA soil respiration chamber system: Winter 2001
Develop robotic positioning system: Winter 2001
Beta test sampling with lightweight IRGA and robotics: March 2002
Set up field experiment with multi-scale, lightweight IRGA, robotics and instrumentation for monitor controlling factors at 2-3 sites: April/May 2002
Analyze data: June 2002
Develop extensive sampling protocol: July 2002
Evaluate potential for use in NPP assessment of LTEP analysis: July 2002
## Estimated Budget

<table>
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<th>Description</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salary</td>
<td></td>
</tr>
<tr>
<td>Nay – 0.5 FTE (partly covered by SEP Team funds)</td>
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</tr>
<tr>
<td>Bormann – 0.1 FTE (partly covered by SEP Team funds)</td>
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<td>Kiester – 0.05 FTE</td>
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<tr>
<td>Technicians, 2 for 2 months @ $10.00/hr</td>
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<td>Travel and supplies</td>
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<tr>
<td>Travel to field sites (depends on sites)</td>
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<tr>
<td>Lightweight soil respiration systems: 3 @ $4000 ea.</td>
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<td>Consulting</td>
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<tr>
<td>Robotic Apparatus</td>
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<tr>
<td>Soil analyses</td>
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<td>Total</td>
<td>$80,000</td>
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</tbody>
</table>

Sources of funding include the SEP Team budget (part of salaries), the LTEP program coordinated between the ECOP and Roger Clark and Robyn Darbyshire (new combined PNW Program), and possible other PNW collaborators. Our intent is to use results from this study to develop a larger study funded by NSF or USDA Competitive Grants.

## Literature


Appendix

Li-Cor LI-800 IRGA Specifications
http://env.licor.com/Products/GasAnalyzers/li800/li800.htm

Measurement Principle: Non-dispersive Infrared

Measurement Ranges
14 cm Optical Path: 0-1,000 or 0-2,000 ppm
5 cm Optical Path: 0-5,000 or 0-20,000 ppm

Maximum Gas Flow Rate: 1 liter/minute (with user-supplied pump)

Analog Outputs: 4-20mA, 0-0.5V, 0-1V, 0-2.5V, 0-5V

Power Consumption 0.3A @ 12V average after warm-up

Power Consumption 1A @ 12V max during warm-up

Supply Operating Range: 12 - 30 VDC

Operating Temperature Range -25 °C to +45 °C

Relative Humidity Range 0 - 95% RH, non-condensing

Dimensions 22.2 x 15.2 x 7.6cm; 8.75 x 6 x 3 inches

Weight 1.0 kg; 2.2 lbs.