3 · Experimental design: scaling up in time and space, and its statistical considerations

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3.1 INTRODUCTION

Accurate measurement of the soil CO_2 efflux is critical for the assessment of the carbon budget of terrestrial ecosystems, since it is the main pathway for assimilated carbon to return to the atmosphere, and only small changes in the soil CO_2 efflux rate might have important implications on the net ecosystem carbon balance. Due to this central role in the terrestrial carbon cycle, soil CO_2 efflux has been measured throughout all biomes, and covering all principal vegetation types. Using simplified regressions of soil CO_2 efflux measurements reported in the scientific literature, the total amount of carbon emitted as CO_2 by soils worldwide has been estimated at approximately 68–80 Pg (1995; Raich *et al.*, 2002), representing the second largest carbon flux between ecosystems and the atmosphere. This amount is more than ten times the current rate of fossil fuel combustion and indicates that each year around 10% of the atmosphere's CO_2 cycles through the soil (Prentice *et al.*, 2001). Thus, even a small change in soil respiration could significantly intensify, or mitigate, current atmospheric increases of CO_2, with potential feedbacks to climate change. In fact, soils store more than twice as much carbon globally than the atmosphere (Bolin, 2000) and consequently contain a large long-term potential for the carbon cycle climate feedback. Applying results from small-scale experiments to larger areas is necessary in order to understand the potential role of soils in sequestering or releasing carbon under changed climatic conditions, and to inform management and policy makers about likely consequences of land-use changes on carbon fluxes and stocks in specific regions. As in the example of the global estimate mentioned above, there is inevitably a need to estimate the soil CO_2 efflux over vast areas that it is impossible to cover appropriately by actual measurements, and for time scales beyond the scope of measured data, particularly where future predictions are required.

Scaling up from sparse and infrequent measurements to the level of, for example, catchment, region or even continental or global scales, bears a considerable degree of uncertainty, making such extrapolations difficult. The scope of this chapter is to introduce a range of requirements that are critical to facilitate meaningful extrapolation of results observed on small scales to allow making estimates of soil CO_2 efflux over larger areas and longer time scales. The aim is to provide a general overview in order to enable the reader to design a suitable measuring strategy towards such extrapolations, mainly at the plot and landscape scale.

Measuring techniques can be broadly divided into (1) chamber-based, (2) soil profile and (3) eddy covariance approaches. Chamber-based measurements provide by far the majority of published results, and the general considerations of heterogeneity are valid for all measuring approaches. We therefore concentrate on chamber-based measurements to illustrate experimental designs for dealing with natural variations in soil CO_2 efflux. Soil profile methods allow a vertical resolution of the origin of surface flux contributions, thus providing critical insight into carbon allocation within soils by roots and contributions to the heterotrophic flux component for different soil depths. However, soil profiles inherently create considerable disturbance both during installation and sampling (Fang and Moncrieff, 1998) and are difficult to replicate within plots. For successful applications of this technique, please refer to Tang *et al.* (2003), Liang *et al.* (2004) and Davidson *et al.* (2006). Eddy covariance has been applied to measure soil surface CO_2 flux with some success (see e.g. Law *et al.*, 1999; Janssens *et al.*, 2000; Wilson and Meyers, 2001; Subke and Tenhunen, 2004). This technique has the advantage of causing no disturbance to the soil, but it is restricted to conditions of sufficient atmospheric turbulence, and homogeneity of the surface in the up-wind fetch. However, eddy covariance is much less suited for measuring under a closed forest canopy or in complex terrains.

Although laboratory incubations are important in addressing certain hypotheses (e.g. temperature sensitivity of the heterotrophic component), this chapter does not provide in-depth detail on this topic but concentrates on the relative strengths and limitations of some laboratory-based approaches in the context of flux measurements. References given in that section (and those by Reichstein and Janssens in Chapter 11 of this book) are intended to guide the reader to look up individual studies on technical issues. Soil CO_2 efflux measuring equipment and auxiliary measurements at experimental sites have been addressed in the previous chapter, and the actual methods of scaling and interpreting soil efflux observations with models from laboratory to global scales are covered in Chapter 11. Here we point out some further measurement considerations in relation to capturing temporal variability accurately. Finally, we provide a logical framework of how to design and perform statistically sound experiments for testing hypotheses.

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3.2 SPATIAL AND TEMPORAL VARIABILITY

3.2.1 Sources of variability

Soil CO_2 efflux is the sum of respiratory activity from a variety of sources. Mineralization of carbon from both fresh litter and older soil organic matter (SOM) through soil-dwelling animals, fungi and bacteria comprise the heterotrophic flux contributions. The separation of this flux from autotrophic sources is ambiguous (see Moyano *et al.*, Chapter 7), as definitions in the literature differ according to a classification by the source of carbon being respired and the fraction of soil biota in which respiration actually occurs. Growth and maintenance respiration by plant roots represents the true respiration by autotrophs, but mineralization of carbon contained in compounds secreted by living roots (exudations, mucilage or sloughed root cap cells) by soil bacteria or fungi form a grey area in the categorization of flux origin (see e.g. Kuzyakov, 2006a, b and Högberg *et al.*, 2006 for a recent debate on the issue of separating these flux contributions). For the purpose of this chapter, we consider the portion of soil CO_2 efflux caused by the input of carbon from roots as autotrophic respiration. It therefore includes respiration by mycorrhizal fungi, which obtain substrate for their metabolism nearly exclusively from their hosts' roots, and that of all other soil microbes metabolizing recent plant-derived carbon (such as usage of root exudates). In addition to these biotic flux sources, soils may have a varying degree of inorganic fluxes through the weathering of carbonates contained in soil and underlying geology.

Soils form over periods of hundreds to thousands of years, and their structure and carbon content is mainly a result of the geologic parent material (e.g. bed-rock of varying weatherability, or mineral deposits such as sands or clays), geomorphological conditions (e.g. slope and aspect of the soil surface), local climate and vegetation cover (Jenny, 1980). It is important to note that in particular climate and vegetation cover are not constant site factors but may vary considerably during pedogenesis. Thus organic carbon within the soil represents a mixture of ages, ranging from very recently fixed litter carbon to humified materials literally thousands of years old. This mixture represents not only present site conditions but also a long legacy of previous biotic and abiotic influences. Physical structure and chemical composition of the soil is therefore also linked to the diversity of organisms to which the soil has been a habitat over these periods, with significant implications for the physical distribution of organic matter and cycling of nutrients, which in turn impacts on the vegetation cover above ground (see Chapters 9 and 10).

Consequently, in a physically complex structure such as soil, sources of carbon substrates (for all types of respiration) are not distributed homogeneously, and their availability at any given place may also change with time. Nunan *et al.* (2002) observed different spatial structures in the distribution of soil microbes, which were closely linked to pore space within the soil. While topsoil distributions showed a pattern on the micrometre scale, in the subsoil an additional but separate centimetre to metre scale could be observed.

The abiotic soil environment (e.g. soil temperature, water content, CO_2 and O_2 concentrations) strongly influences the rate at which CO_2 mineralization from different sources occurs. In the literature most attention has been attributed towards soil temperature and

moisture as they impact strongly on both autotrophic and heterotrophic activity. For example, soil temperature fluctuation at the soil surface propagates into soil depths both with dampened amplitude and increasing time lag, which has to be considered if a significant portion of the surface CO_2 efflux originates from a lower soil depth (see also Chapter 11). Soil surface temperature fluctuations are, in turn, strongly dependent on vegetation cover and its exposure throughout daily and seasonal cycles. Irregular canopies, for example, result in considerable differences of incident light at ground level with potentially significant consequences for soil temperatures. Soil moisture conditions may also differ at a small scale, as canopy throughfall often consistently differs in space due to canopy structure or patchiness of ground vegetation cover. Under well developed regular canopies with high leaf area index (LAI) values, by contrast, soil temperature and moisture are likely to be more homogeneous, so that differences in soil CO_2 efflux are likely to be smaller.

There are, however, other important biotic factors, which, although mostly overlooked, should be considered when measuring and modelling soil CO_2 efflux, such as the distribution of live roots and soil fungi, both showing considerable spatial and temporal variations at the plot scale. Root density is affected by soil structure (e.g. bulk density and rock content) and soil depth, and the distribution of nutrients and water availability at the site. Furthermore, mycorrhizal fungal hyphae are important structures for the bidirectional translocation of nutrients from local patches ('hot spots') to roots, and of carbon from the host plant to the fungus for its growth and metabolic requirements (Smith and Read, 1997). Naturally, this flow of carbon from plants to symbiotic fungi is directly linked to the rate of assimilation by the canopy, and as such will show seasonality due to canopy phenological changes. Therefore, the degree to which biotic conditions differ within a site is strongly dependent on local abiotic conditions, small-scale topography and site management history. It is also important to consider the degree of heterogeneity throughout the phenological cycle, as a single area survey may not capture the variation of this biotic flux contribution accurately. As the autotrophic flux component of the biotic flux might be largely independent of the commonly observed changes in soil temperature, due to temperature acclimation of root respiration (Atkin *et al.*, 2000) or mycorrhizal hyphae (Heinemeyer

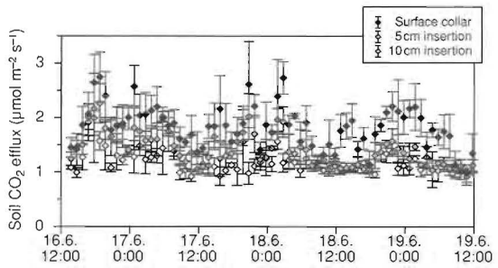

Figure 3.1 Diurnal variation in mean hourly soil CO_2 efflux measured in a 15-year-old temperate pine forest (Heinemeyer *et al.*, unpublished) using different collar insertion depths of 0 cm (surface collar, black), 5 cm (grey) and 12 cm (white). Soil respiration was measured with a multiplexed long-term monitoring system (Li-Cor 8100, Li-Cor, Nebraska). Collar insertion depth is measured from the litter surface and therefore includes the O_l, O_f layer of about 2 cm. Symbols indicate hourly mean fluxes with error bars of 1 SE (n = 3) over the period of four consecutive days during summer 2006, one week after collar insertion.

et al., 2006, 2007), it might be necessary to monitor additional factors such as plant phenology and root activity for accurate up-scaling procedures. In fact, the role of the autotrophic flux component may have been largely underestimated in the past as soil collar insertion even of only a few centimetres might have cut off a large fraction of the autotrophic carbon supply to fine roots (as shown by Wang *et al.*, 2005) and their associated mycorrhizal hyphae, predominantly living in the top organic rich soil layers. Figure 3.1 shows a reduction in measured surface CO_2 flux with increasing soil collar insertion depth in a 15-year-old pine plantation without any ground vegetation. A considerable loss of respired CO_2 could be observed for the relatively shallow depth of 5 cm (which includes 2 cm of the surface litter layer), and also appears to reduce diurnal variations and overall variation between replicates (i.e. standard error). The shown flux reductions were still maintained six months after the collar insertion (data not shown). Figure 3.1 therefore clearly shows that where soil CO_2 efflux is measured from soil collars, these should be as shallow as possible. It also indicates that the commonly employed 'good practice' of measuring fluxes from collars installed at least 24 hours before measurements is not sufficient to allow natural efflux conditions to re-establish. Good seals with the soil surface can generally be achieved with

Table 3.1 *Coefficients of variation (CV) for spatial variation within forested sites reported for different ecosystems.*

	CV	Reference	Comments
Boreal forest	18–45%	Pumpanen *et al.* (2003)	CV found to increase with magnitude of CO_2 efflux
Boreal forest	87%	Rayment and Jarvis (2000)	
Temperate hardwood	30%	Davidson *et al.* (2002)	
Temperate coniferous	28%	Yim *et al.* (2003)	*Larix* plantation
Temperate coniferous	40%	Buchmann (2000)	CV of peak rates in four *Picea* stands of different ages
Temperate coniferous	42%	Subke *et al.* (2003)	Measured in one of the stands covered by Buchmann (2000)
Mediterranean deciduous	40%	Tedeschi *et al.* (2006)	Oak coppice
Tropical forest	30%	Davidson *et al.* (2002)	
Tropical pasture	30%	Davidson *et al.* (2002)	

quite shallow collar insertions. Where this is not possible (e.g. in the absence of a humus layer with relatively brittle mineral soil exposed at the surface), fine roots are likely to be less concentrated in the top soil layer.

3.2.2 Coping with variability

3.2.2.1 Spatial variability

The previously described sources of biotic and abiotic drivers of soil CO_2 efflux result in the naturally observed spatial soil CO_2 efflux variations. Sites that have experienced recent physical disturbance and have a poorly developed canopy are likely to have significantly more variability than mature stands, while agricultural sites where soils have been homogenized, for example by ploughing, will show a lesser extent of variability. Table 3.1 lists the coefficient of variation (CV = standard deviation/mean soil CO_2 efflux) as a measure of the variability between sampling points in a range of ecosystem types reported in the literature.

Values for the CV of around 40% are commonly observed, and the number of sampling locations required to produce a reliable estimate of the actual soil CO_2 efflux value is directly dependent on the degree of variability at a given site. Once the degree of variability within a stand has been established, the number of sampling points (n) that will produce an estimate within a desired range of the true value for a given probability level is $n = \left[\frac{z_{\alpha/2}\sigma}{D}\right]^2$, where $z_{\alpha/2}$ is the critical z-value that is at the vertical boundary for the area of $\alpha/2$ in the

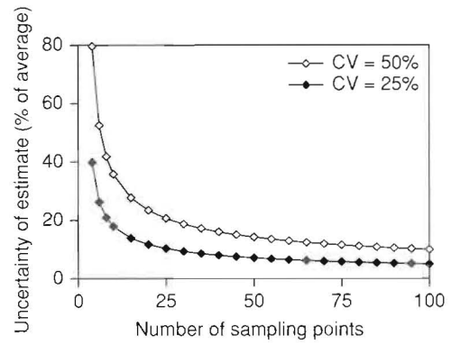

Figure 3.2 Effect of the number of sampling points within a stand on the uncertainty of a spatially averaged soil CO_2 efflux for a confidence level of 0.05. The degree of uncertainty shows a steep decline as the number of sampling points increases to about 20, and increases directly with the magnitude of the coefficient of variation (CV).

right tail of the standard normal distribution, σ is the standard deviation and D is the desired range of the true efflux value (e.g. 20%). Figure 3.2 illustrates the effect of both the number of sampling points and CV on the degree of uncertainty in a spatially averaged flux, based on this relationship.

Two studies applying this analysis to extensive datasets (mixed temperate hardwood forest by Davidson *et al.* (2002), and *Larix* plantation by Yim *et al.* (2003)) showed that for a CV of *c.* 30%, 8 to 10 sampling points are required to reach 20% of the true site CO_2

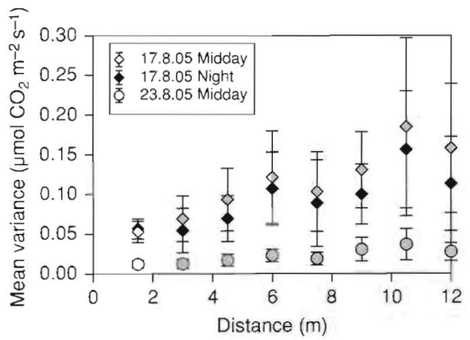

Figure 3.3 Effect of sampling distance on mean soil CO_2 efflux (μ mol m^{-2} s^{-1}) at a UK heather moorland site (Heinemeyer *et al.*, unpublished). Mean variances are based on soil respiration measured from eight locations with 1.5 m spacing using a Li-Cor 8100 system (measured on 20 cm deep PVC collars; error bars indicate standard errors for the calculated variances). The three symbols reflect three different measurement periods in August 2005: two during the 17th (dry and warm) and one on the 23rd (wet and cold). Note the reduced variance due to very low fluxes after waterlogging on the 23 August.

efflux at a 95% confidence level, while 30 to 40 sampling points are required for estimates to be within 10% of the site mean at the same level of confidence. Another commonly overlooked issue is the spatial autocorrelation, i.e. the closer the flux sampling points to each other, the more similar are the expected soil CO_2 effluxes. This behaviour can be analyzed by geostatistical variogram analysis (Cressie, 1993) and should be accounted for in the sampling design by placing sampling points far enough from each other to guarantee statistical independence and to avoid pseudo-replication (Hurlbert, 1984). Figure 3.3 illustrates the degree of spatial heterogeneity at a moorland flux site in England (Malham Tarn) where soil CO_2 efflux has been measured along a transect with regular collar spacing of 1.5 m. The mean variance for a given collar distance (i.e. multiples of 1.5 m in this case) can be calculated according to: $\gamma(d) = \frac{1}{2n}\sum(R_x - R_y)^2$, where γ is the mean variance (i.e. a measure of the similarity) between collars, d is the distance between collars, n is the number of pairs of observations in any of the distance classes and R is the soil CO_2 efflux measured on any two collars (x and y).

3.2.2.2 Temporal variability

Owing to the natural fluctuations in biotic and abiotic drivers of soil CO_2 efflux, observed rates commonly show a pronounced seasonal and diurnal variability. Studies aiming to quantify soil CO_2 efflux over longer periods have to ensure that all key efflux situations (e.g. summer drought, rewetting, budburst etc.) are well represented by the sampling strategy. Thus the sampling frequency needs to allow a meaningful interpolation of measurements in order to adequately describe the total integrated soil CO_2 efflux. However, as with capturing the spatial variability, this requirement is most commonly limited by the cost of materials or labour involved. Additional bias may be introduced if soil CO_2 efflux is always sampled at the same time of day, missing out key biotic (e.g. diurnal changes in autotrophic activity) and abiotic (e.g. lag in soil temperature changes with depth) components.

Soil CO_2 efflux is strongly correlated over time, and while there is usually a pronounced diurnal variability in surface fluxes, these tend to show relatively small changes between successive days. Fluxes measured from the same location after only a short time interval are therefore not independent observations and may confound the statistical analysis in an experiment. Semivariance analysis is a useful tool to analyze the degree of autocorrelation over time and helps to determine the adequate sampling interval in order to avoid oversampling. Figure 3.4 illustrates the degree of correlation between soil surface CO_2 fluxes with an increasing time lag. The graph shows local minima between fluxes at the same time of day (i.e. time lag of multiples of 1 day), with a general increase in variance over the first 5 days. Thereafter, variances between efflux measurements are relatively constant while still retaining the lowest variance for measurements made at the same time of day. For this particular site, it can therefore be concluded that a periodic sampling strategy with measurements taken at a minimum of 5-day intervals would not oversample and thus prevent autocorrelated results.

To further assess both the impact of sampling interval and potential biases resulting from selective sampling at specific times of the day, soil CO_2 efflux from the same dataset of continuous hourly soil CO_2 efflux data was 're-sampled'. To simulate periodic sampling, fluxes were averaged either for the morning hours

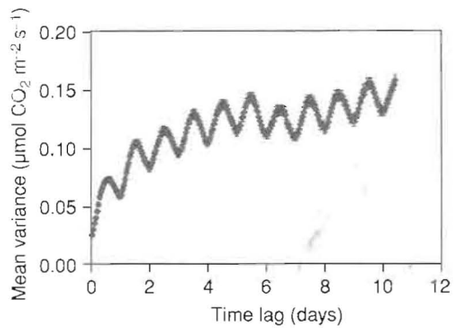

Figure 3.4 Mean variance of hourly soil CO_2 efflux values with fluxes measured from the same locations, but with increasing time lags. Variances were computed for a dataset with hourly flux measurements (disregarding data gaps) over three periods of the growing season (29 April 1999–4 July 1999, 28 July 1999–7 September 1999 and 29 October 1999–2 December 1999) in a mature temperate spruce forest (see Subke *et al.*, 2003). The complete dataset includes 3476 hourly flux measurements, allowing variances to be calculated for between 3344 (interval = 1 hour) and 2324 (interval = 10 days) pairs of flux values. Error bars indicate standard errors for the calculated variances.

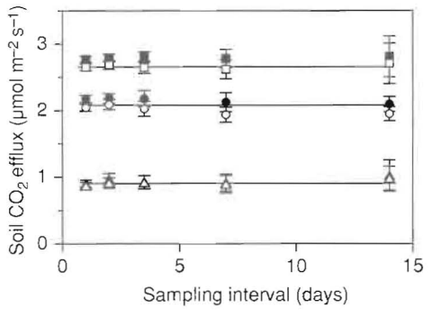

Figure 3.5 Effect of re-sampling a continuous dataset of soil CO_2 efflux measured in a mature temperate spruce forest (see Subke *et al.*, 2003) using different sampling frequencies. Symbols indicate mean fluxes with error bars of 1 SE, while horizontal lines indicate the mean flux obtained from the continuous dataset. Circles: 29 April 1999–4 July 1999; squares: 28 July 1999–7 September 1999; triangles: 29 October 1999–2 December 1999. For each period, open symbols are average fluxes measured between 9 a.m. and 1 p.m., while solid symbols are fluxes measured between 9 a.m. and 6 p.m.

(9 a.m.–1 p.m.), or for daytime measurements (9 a.m.–6 p.m.), for 1 day, 2 days, bi-weekly, weekly or fortnightly sampling intervals. The analysis shows that increasing the sampling interval results in increasing deviations from the continuously measured average (which for the purpose of this analysis is assumed to represent the true site efflux), reaching values of up to 10% (Fig. 3.5). The error bars in Fig. 3.5 indicate the lower degree of certainty of low frequency measurements owing to the smaller number of sampling dates. Parkin and Kaspar (2004) report a similar increase in cumulative CO_2 flux estimate with increasing lengths of sampling intervals.

Figure 3.5 further shows a small but consistent bias resulting from the different periods within a day over which samples were collected. At this particular site, soil CO_2 efflux showed a slow increase after sunrise, following the temperature increase in the soil. Peak values were commonly observed in the early afternoon and fluxes declined slowly before dropping after sunset. In this example, fluxes measured between 9 a.m. and 1 p.m. were a better representation of the actual site mean efflux than those collected between 9 a.m. and 6 p.m. Pair-wise comparison of fluxes in the first sampling period of Fig. 3.5 showed a significant difference between fluxes obtained during the morning and those obtained throughout the daytime hours ($t = 7.21$, $p < 0.001$), while neither of the estimates differed significantly from the true 24-hour mean of corresponding sampling days. On average, morning fluxes underestimated 24-hour means by 3%, while daytime hour flux estimates overestimated the true diurnal mean by the same margin. Notably the time lags in the diurnal soil CO_2 efflux dynamics differ according to site conditions and the relatively small error introduced by either morning or daytime sampling in this example cannot be automatically assumed to hold for different sites. Data in Fig. 3.1, for example, show peak values at around midnight, which is likely to be due to the time lag for assimilation products fixed throughout the day to reach the roots and rhizosphere.

Correcting any possible bias resulting from the time of day during which sampling took place may be possible if the diurnal variation of soil CO_2 efflux (meaning day- and night-time fluxes) is measured repeatedly throughout the measuring period. If the bias is constant throughout the period, a simple multiplicative correction may suffice; alternatively, a simple soil temperature model may be necessary to correct fluxes.

Table 3.2 *Comparison of attributes for automated and manually operated soil CO_2 efflux measuring systems. Please note that there is a considerable variety of measuring principles, so that within each of the two categories individual aspects may vary.*

	Automated system	Manual system
Measuring frequency	continuous	periodic
Number of sampling points	small	high
Technical requirements	high	low
Labour intensity	low	high
Capture of spatial heterogeneity	low	high
Capture of temporal heterogeneity	high	low
Suited for	Time series analysis Capture of 'events'	Areal survey

3.2.2.3 Implications for soil CO_2 efflux sampling strategies

Soil CO_2 efflux measurements using chambers in the field are commonly done by either continuous automated systems or manually operated chambers with measurements carried out in periodic campaigns (see Chapter 2 Pumpanen *et al.* for a more detailed description of measuring methods). The choice of a measuring system depends principally on the objective of an experiment. Table 3.2 provides a general overview of the attributes of automated continuously measuring systems and those of manually operated systems. However, while these attributes are generally correct, there is considerable variability within each category. A further constraint is commonly posed by the availability of resources to invest in either materials or labour, which are assumed to be restrictive for this comparison. Given a big enough budget, it is feasible to either measure with a continuous system from a high number of sampling points or to measure fluxes manually at high frequency, thus compensating for some of the aspects highlighted in Table 3.2.

In their investigation into trade-offs between the resolutions of either measuring mode, Savage and Davidson (2003) conclude that the manual mode is beneficial for investigations where the mean soil CO_2 efflux of a site is under investigation, with significant reductions in the 95% confidence intervals owing to the better capture of spatial heterogeneity. However, this sampling mode was not well suited for capturing short-term changes in soil CO_2 efflux, for example following wetting events or changes in temperature. Studies interested in empirical modelling of soil CO_2 efflux to environmental factors would therefore benefit from data obtained from automated continuous measurements. A combination of both approaches is advisable in order to avoid bias due to the shortcomings of either temporal or spatial representation.

Experimenters operating continuous systems with low spatial replication are well advised to first assess spatial heterogeneity with a survey chamber in order to test how representative the continuous sampling locations are. Again, this survey should ideally be repeated throughout the annual cycle if measurements are to be used for extrapolation of annual fluxes.

3.2.3 Laboratory measurements

Laboratory incubations of soils allow a close investigation of the respiratory response to specific environmental parameters (most commonly temperature and soil moisture), or soil amendments with respiration substrates, nutrient solutions or pollutants (Dilly and Nannipieri, 2001; Allen and Schlesinger, 2004; Miller *et al.*, 2005; Smith, 2005; Shaver *et al.*, 2006). The obvious advantage is the level of control over a range of parameters (both biotic and abiotic) influencing soil CO_2 efflux under field conditions, allowing a clearer interpretation of results from experimental treatments. Depending on the experimental aims, soil samples from the field may

be left intact as complete monoliths or separated into different soil components (surface litter, organic horizon(s), mineral soil and roots). Soil extraction from the field and incubation in the laboratory by its very nature represents a major disturbance. Even if soil cores are left intact, biological processes within this portion of soil are significantly affected by the physical disturbance during extraction and interruption of the autotrophic connections (i.e. roots and mycorrhizal hyphae). Depending on the mode of soil sampling, local compaction or loosening of the soil matrix is possible, with considerable influence on soil diffusivity due to artificial changes in soil pore space volumes. Roots that were severed are likely to lose labile organic compounds ('wound respiration') in the short term (Cabrera and Saltveit, 2003), while the obvious lack of carbon input from the plants and subsequent loss of exudations from roots within the soil core means that substrate supply to a host of microbial organisms have been removed. The result is a rapid decline in soil CO_2 efflux in the initial period (on the time scale of hours to a few days) following soil extraction in the field (Reichstein *et al.*, 2005). Ultimately, roots (and any other directly dependent organisms such as the mycorrhizal mycelium) within the core will die, so that the amount of dead biomass is artificially increased with respect to soil conditions at the site the sample was taken from.

Laboratory incubations of root-free soil, on the other hand, can be used to estimate the carbon mineralization potential of different soil parts or the microbial heterotrophic response to temperature and soil moisture conditions. Due, again, to the inherent disturbance by the sampling process and subsequent separation of soil components, there is a clear limitation to the possibility of extrapolating soil CO_2 efflux obtained in laboratory incubations to field conditions. For investigations aiming at quantifying the soil CO_2 efflux under field conditions or addressing any hypotheses involving an intact autotrophic flux component, measurements made on laboratory incubated soil samples alone are not suitable as an experimental approach. However, soil CO_2 efflux studies based on laboratory incubations have been instrumental in supplementing field-based measurements by separating out individual aspects of soil CO_2 efflux responses to environmental conditions (Fang *et al.*, 2005; Miller *et al.*, 2005; Reichstein *et al.*, 2005), the potential of CO_2 being mineralized from different forest sites (Person, 2000; Sjoberg *et al.*, 2004), as well as investigations of the stability of soil organic matter fractions (Franzluebbers *et al.*, 2001; Ladegaard-Pedersen *et al.*, 2005; Leifeld and Fuhrer, 2005) or effects of pollution and soil amendments on soil microbiota (Rajapaksha *et al.*, 2004; Fuentes *et al.*, 2006; Oorts *et al.*, 2006).

3.2.4 Scaling up

Scaling up in space and time is always based on the generalization of the data with respect to factors controlling the variation. Day-to-day and seasonal variation in time is often largely dependent on temperature, soil moisture and simple measures of vegetation activity (such as leaf area index) and can be modelled relatively easily. The longer the time scale, however, the more interacting factors come into play (e.g. carbon pool dynamics, disturbances – including small non-visible ones – and population dynamics), reducing our ability to predict longer term cycles and trends in soil efflux. Similarly, spatial variation can be modelled quite well along gradients where temperature, soil moisture regimes and vegetation productivity are the dominating factors (e.g. along continental gradients) (Reichstein *et al.*, 2003). As soon as those factors are less dominant, subtler but important factors might come into play: prominently soil chemical status (e.g. pH, nutrients), vegetation cover and site history (Reth *et al.*, 2005). There is still no general picture of how these factors co-determine the between-site variation of soil respiration. Consequently, scaling up is difficult and depends largely on well stratified sample databases. Typical models addressing temporal and spatial variation at different scales are discussed in Chapters 11 and 12.

3.2.5 Site variation: random, stratified or systematic design, and avoiding bias

Apart from sampling soil CO_2 efflux from a sufficient number of locations according to a site's heterogeneity, the allocation of adequately spaced sampling points (see Section 3.2.1 for autocorrelation issues) is equally important in order to achieve a representative estimate of the true soil CO_2 efflux value. An appropriate design will vary according to the site conditions and depends on the question to be answered. Mainly there are three types of sample design: (1) random, (2) stratified or (3) systematic. Whereas (1) assumes fairly uniform site

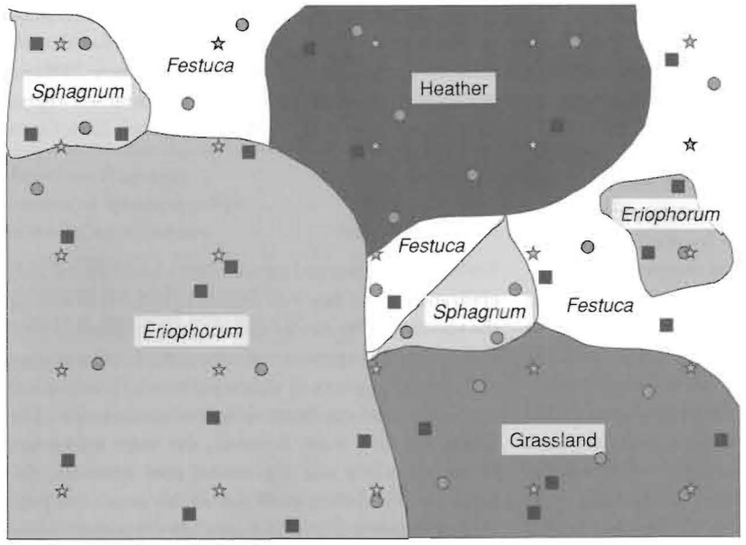

Figure 3.6 Example of three different sample designs for assessing CO_2 efflux site variability in a heather moorland with a patchwork, dominant vegetation cover. Symbols indicate the three sets of 25 measurement locations each: squares and circles represent the random and stratified design, respectively, whereas a systematic approach would cover the area in 25 equally spaced points (stars).

conditions (e.g. old beech forest on brown earth), (2) is more suitable for sites with known spatial variability (e.g. heathland covering a patchwork of soil types with differences in dominant vegetation types and slope variation). Systematic sampling (3) might be considered as straightforward but ignores underlying site variability. However, it is suitable to answer questions such as variance distance relationships (see Section 3.2.1 for semivariogram-based analysis). More information on sampling approaches is given in Hurlbert (1984).

From Fig. 3.6 it is clear that using different sample strategies will lead to different results. In a first approach we will only consider one environmental factor, vegetation cover, in order to obtain an 'overall site soil flux' estimate. In our case (Fig. 3.6), the random design will not only misrepresent the patchwork of dominant vegetation types, it will also lead to a bias towards *Eriophorum* coverage (11 sample points vs. only 3 for *Sphagnum*). Both would be much better accounted for by a stratified design (5 sample points for each vegetation zone). To make sure there is no further bias introduced, the stratified design needs to be allocated in a randomized way, i.e. sample points should be given coordinates based on a random number approach within each stratified zone (e.g. five random coordinates for each vegetation type). However, in another approach one might want to focus on assessing the 'dominant site soil flux' allocating more measurements to the dominant vegetation type, thus the stratification must be weighted according to the proportion of the total area occupied by each vegetation type.

A different approach altogether would be to include many more environmental variables, which would demand more sophisticated geostatistical methodology such as sampling of assembled data, for which examples are given in McBratney *et al.* (2003). For example, a constrained Monte Carlo sampling scheme selects μ different values from each of the different variables by dividing them into several non-overlapping intervals on the basis of equal probability. One value from each interval is selected at random with respect to the probability density in the interval. The obtained μ values are then paired in a random manner between the many environmental variables until μ-duplets are formed; searching through the data can then find the locations that are taxonomically most similar to the combination of values chosen (e.g. heather on deep peat), or find locations that match the intervals in the various variables (e.g. pH ranges). In either case, this will result in a set of μ spatial coordinates (locations) for observation (see McBratney *et al.*, 2003). There are many other sophisticated geostatistical procedures and practical considerations, and the reader may want to consult McBratney *et al.* (2003) for useful examples on related geostatistical methods such as Kriging and co-Kriging. Kriging is a process by which values are estimated at those locations that have not been sampled. The technique uses a weighted average of neighbouring samples to estimate the 'unknown' value at a given location, which can be optimized using the semivariogram model. The technique also provides a 'standard error', which may be used to quantify confidence levels. Co-Kriging uses a similar interpolation technique but estimates map values if the distribution of a secondary variable can easily be sampled more intensely than the primary variable.

3.2.6 Using geographical information systems (mapping and querying)

An alternative to the complex geostatistical procedures mentioned above is the use of a geographical information system (GIS), which can help considerably with the development of field sample strategies. In our example, the stratified sample design locations shown in Fig. 3.6 might change considerably if sample point allocation is weighted on a vegetation type area basis as done by Garnett *et al.* (2001) for soil sampling. This weighted allocation will reflect the soil fluxes under different vegetation covers in proportion to their area, thus providing an undistorted mean flux estimate for the entire site. There are many GIS software packages available offering different levels of complexity and user knowledge, and the reader may wish to consult specific literature. In many cases a wide variety of plot or landscape information (e.g. soil and vegetation types, soil pH, organic carbon content, slope and soil depth) is available about a given area on which sample strategy can be based. However, it will become increasingly difficult to display and query those data in conventional software in order to assist with sample design. In a GIS such digitized data are then imported as either polygons (areas) or point information that can then be used to draw map layers and to query any combination of layers. For example, the soil type in Fig. 3.6 might actually not overlap with the dominant vegetation or there might be steep slopes across the heather and grassland communities, both might strongly impact on the measured soil CO_2 efflux. In a GIS a query can be done, outlining different zones based on all the information available (e.g. including slope grades), on which a more accurate stratified design can be based. The intention would be to sample the reference area as outlined above in order to fit a model and extrapolate to the rest of the area. This might give a better chance of fitting local relationships with a given sampling effort, and should be more efficient in required field time.

The GIS approach may also help with the spatial display of soil fluxes and to model point measurement integration (e.g. plot interpolation, see Kaye and Hart, 1998), which can be done using quite a diverse set of procedures (e.g. surface or grid interpolation making different assumptions about spatial relation). Further, if larger than plot scale information is available, such as land use, vegetation or soil maps, then scaling up the integrated plot results to the landscape is achievable in a GIS using spatial information, as done by McBratney *et al.* (2000) for soil mapping.

3.3 FORMULATING AND TESTING HYPOTHESES

Whereas the previous part of this chapter provides critical knowledge for observation-based science (e.g. obtaining meaningful spatial and temporal site flux variations) the following also considers theoretical and practical issues related to experimental manipulation (e.g. hypothesis testing). The basis of science is the formulation and testing of hypotheses by applying experimental treatments, which distinguishes it from purely observational disciplines such as natural history or even assessing temporal and spatial flux variability as outlined previously. It is assumed that the null hypothesis is true and the scientist will look for evidence in the data to either support or reject the null hypothesis. A fundamental concept of the method is to assume that the null hypothesis is true until there is overwhelming evidence against it (typically, less than a 1% or 5% chance of obtaining the observed value or one more extreme if, in fact, the null hypothesis were true).

However, it is not always easy to formulate clear and testable hypotheses or design a balanced experiment with appropriate controls. Therefore, care should be taken to follow certain guidelines, which will lead to successful experimental testing of hypotheses and thus

provide meaningful answers. In the following section we suggest an experimental step-by-step approach as a basis for scientific hypotheses testing, which can be summarized in five steps.

1. Make the **observation**.
2. Formulate the **hypothesis**.
3. Draw the **graph**.
4. Design and perform the **experiment**.
5. Evaluate data with the appropriate **statistical design**.

Although the following section will be sufficient in a soil respiration context, there might be additional precautions needed to ensure successful hypothesis testing under special circumstances (e.g. when measuring in unusual environments). The most common mistakes are made by having (1) an inappropriate or no control treatment at all or (2) no pre-treatment data; this five-step approach is intended to prevent such mistakes.

3.3.1 Make the observation

Soil respiration data are used to inform models about site-specific soil CO_2 efflux behaviour throughout the year in order to improve model performance (see Chapter 11 Reichstein and Janssens). As explained above, the annual cycle might be divided into several key soil respiration process stages (e.g. snow cover, thawing, bud burst etc.). Thus different observations throughout the year might lead to addressing different hypotheses. For example, the observation might be that winters with less snow cover result in comparatively low soil respiration flux as observed by Monson *et al.* (2006). One might link this to better soil insulation under snow cover, leading to warmer soil temperatures and thus higher microbial activity, or protecting roots from frost damage. However, the observation needs to be tested scientifically; it is not enough to compare one year with another as other factors leading to higher soil respiration fluxes might have changed as well, which crucially remained unobserved.

3.3.2 Formulate the hypothesis

The hypothesis based on the above observation can be phrased as: 'Soil CO_2 efflux increases with depth of snow cover'. The null hypothesis that is going to be tested statistically therefore states: 'Higher snow cover depth does not result in higher soil CO_2 efflux'.

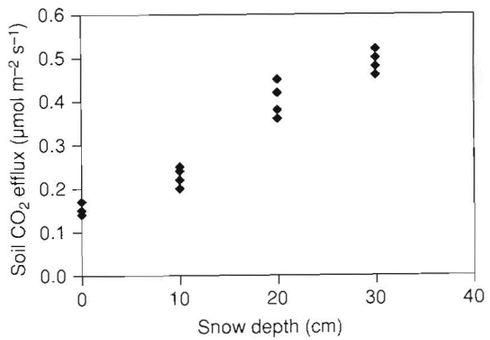

Figure 3.7 Hypothetical graph illustrating the expected correlation between soil respiration (*y*-axis) measured under snow depths treatments (*x*-axis) on which the hypothesis is based (n = 4). Note that the correlation is expected to be non-linear; consequently flux measurements at more than two snow depths treatments are needed. Further, the dependent variable is placed on the *y*-axis, indicating that soil respiration depends on snow depth and not the other way round.

3.3.3 Draw the graph

A first graph (Fig. 3.7) aims at illustrating the hypothesis – in this case a correlation. It is important to note that drawing the graph at this stage does not reflect a foregone conclusion of the outcome of the experiment. The graph reflects one possible outcome (based on the observations that led to the hypothesis), it is intended as a guide towards the most adequate statistical test.

In this graph we already include a critical aspect for the sampling strategy: as we do not know whether there is a critical snow depth from which the hypothesized insulating effect becomes effective (i.e. a likely non-linear relationship between snow depth and soil respiration rates), we will impose four different snow depth treatments. However, as it is possible that there is a minimum time to produce a reduction in flux activity by frost penetration into the soil, we will have to extend the previous plan (Fig. 3.7) and add repeated flux measurements. Based on this, we may proceed with a second hypothetical graph, which sets out the logistics of the experiments; showing extended fortnightly sampling over 15 weeks (Fig. 3.8). Please note that this example is intended to give a guide to the planning of an experiment; measuring CO_2 efflux from soil snow is a considerable technical challenge (Hirano, 2005; Suzuki *et al.*, 2006) and is not part of this exercise. Given that we

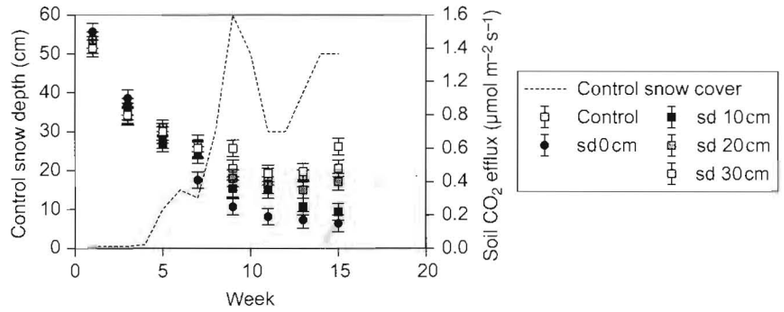

Figure 3.8 A hypothetical graph for the experimental testing of the hypothesis that snow depth is positively related to soil CO_2 efflux. Symbols indicate predicted fortnightly soil respiration fluxes (± 1 SE is an indication that we need more than three replicates!) on the right *y*-axis for the four snow depth (sd) treatments (i.e. snow depth limited to a maximum depth of either 0, 10, 20 or 30 cm by regular sweeping). A hypothetical natural snow cover depth for the unswept control (natural snow cover) is indicated on the left *y*-axis (broken line). Note that the final data (week 15) correspond to the hypothetical data presented in Fig. 3.7.

want to repeat measurements from the same locations, and compare the fluxes from different snow depths, the appropriate statistical test is an analysis of variance (ANOVA) with repeated measures. This also has implications for the sampling strategy as the number of replicates required for each treatment has to be sufficient to yield the statistical power to resolve possible differences. Further, the practical guidance for semivariogram assessment for spatial and temporal flux measurements in order to avoid pseudo-replication should be considered (see Section 3.2.2.1).

By drawing this second graph (Fig. 3.8), and including hypothetical error bars, we are automatically guided to the material requirements of the measuring process and we can instantly recognize if this will, for example, conflict with the capabilities of the measuring system (number of collars available, time required to complete measurements from all locations etc.) or time issues (e.g. holidays). Also note the pre-snow measurements indicated in Fig. 3.8, which are critical to reveal any possible difference in location that is independent of the snow depths. Details such as pre-treatment fluxes, controls and time issues are easily overlooked, and the graph is intended to avoid such mistakes.

3.3.4 Design and perform the experiment

Based on this example, we would plan to measure soil CO_2 efflux from 15 locations beginning well before the first snowfall at fortnightly intervals. These 15 collars are divided into five different experimental groups (four imposed snow depths and one 'control' of natural snow height), using a randomized block design (see Hurlbert, 1984) to ensure that the variances of all groups are identical. In the blocked treatment design, the selected measurement locations are spatially allocated within a block containing a full set of treatments, and blocks are spaced widely enough to avoid pseudo-replication. Blocking can also be used to create a more homogeneous experimental test bed according to similarity criteria, which are ideally based on a pre-treatment ranking (e.g. three blocks with each containing the three plots with highest, medium and lowest soil CO_2 efflux rates). Experimental blocking has two major advantages, both of which increase the statistical power as the block effect can be 'deducted' from the data: (1) it will reduce within-block variability and (2) it can take into account potential environmental gradients (e.g. of soil moisture or pH). As snow depth increases with time, regular sweeping achieves the imposed snow depth of each treatment, and we would plan to continue measurements of CO_2 efflux at fortnightly intervals.

3.3.5 Evaluate the data with the appropriate statistical design

For the statistical analysis, all flux data collected from the time when the snow cover exceeds 30 cm (i.e. after week 8 in Fig. 3.8, in this example) would be considered. Other tests may be considered to look, for example, at

the variation of temperature (in air) and below the different snow depth treatments (in the soil) during the experiment. These factors may be included in the statistics by means of an analysis of covariance (ANCOVA). For more detailed advice on choosing appropriate statistical tests the reader may wish to consider special literature such as Dytham (2003).

3.4 CONCLUSION

Capturing the spatial and temporal heterogeneity of soil CO_2 efflux is one of the biggest challenges to obtaining flux estimates that allow scaling up to larger scales. The aim of this chapter was to introduce the reader to the sources of variability, and to illustrate possible theoretical and practical approaches in order to allow meaningful measurements of complete flux sums. As we have pointed out throughout this chapter, variability of fluxes in time and space is strongly influenced by site-specific conditions and the methodology used. For the purpose of scaling up, it would be desirable to separate individual influences on soil CO_2 efflux, since simplistic parameterizations hold the risk of confounding different sources of variability. While the dynamics of soil CO_2 efflux through the growing season are likely to correlate reasonably well with temperature (and possibly soil moisture), a simple parameterization on these factors alone will likely mask their indirect influence on plant activity, which in turn affects soil CO_2 efflux. A good spatial coverage including the experimental separation of autotrophic and heterotrophic fluxes in the field, and independent parameterization is likely to provide a more meaningful basis for larger scale modelling, where plant activity can be modelled independently, and thus providing a possible input parameter for autotrophic flux contributions. However, any such experimental work needs to be based on a sound statistical design and we hope that our experimental step-by-step approach will be useful to the field scientist responsible for obtaining 'meaningful numbers' on soil carbon turnover processes.

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4 · Determination of soil carbon stocks and changes

Mirco Rodeghiero, Andreas Heinemeyer, Marion Schrumpf and Pat Bellamy

4.1 INTRODUCTION

4.1.1 Soil carbon pools and the global carbon cycle

In terrestrial ecosystems soils represent the major reservoir of organic carbon (Table 4.1), but with large and yet unquantified uncertainties in their estimates (mainly due to low soil sample numbers used for global upscaling and assumptions on mean soil depths). At the global level, the soil organic matter (SOM) pool (estimated to 1 m depth) contains about 1580 Pg of carbon (Pg = 10^{15} g), about 610 Pg are stored in the vegetation and about 750 Pg are present in the atmosphere (Schimel, 1995). Carbon is found in soils both in organic and inorganic forms (Table 4.2). Organic carbon is commonly classified into three 'arbitrary' pools, mostly for modelling purposes (such as in CENTURY), i.e. fast, slow and passive reflecting the rate of turnover. However, it is difficult to relate these pools to soil carbon fractions (see Section 4.1.5). The total amount of carbonate carbon to 1 m depth is estimated at 695–748 Pg carbon (Batjes, 1996). About one third of organic soil carbon occurs in forests and another third in grasslands and savannas, the rest in wetlands, croplands and other biomes (Janzen, 2004). The global soil organic carbon map (Fig. 4.1, ISLSCP II; ORNL DAAC, http://daac.ornl.gov/) shows the areas of high soil organic carbon predominantly in cold boreal (e.g. Northern Canada) and warm and humid tropical regions (e.g. South-East Asia), reflecting areas of deep organic soils (i.e. peatlands). However, Fig. 4.1 also shows that even temperate zones, for example the United Kingdom, can contain considerable amounts of soil organic carbon in wet and cold upland regions.

Most of the soil organic carbon is not inert, but in a continuous dynamic state of accumulation and decomposition (Janzen, 2004; Schrumpf *et al.*, 2008), the schematic soil carbon cycle in Fig. 4.2 indicates this continuous exchange of carbon between the soil and the atmosphere, mostly as carbon dioxide (CO_2) and methane (CH_4). Consequently, any net carbon loss from soils will increase the CO_2 concentration in the atmosphere and in water bodies, whereas net accumulation in soil carbon (or sedimentation in rivers or lakes etc.) can contribute to the reduction of the atmospheric carbon pool (Ellert *et al.*, 2001; Lal, 2004). This cycling of carbon is increasingly influenced by human activities (IPCC, 2007). On an annual basis, global soil respiration estimates amount to about 80 Pg carbon (Schlesinger and Andrews, 2000; Raich *et al.*, 2002), roughly ten times the annual flux from fossil fuel combustion (7.2 Pg carbon; IPCC, 2007). Crucially, past and current cultivation of soils led to significant soil carbon losses of 50 Pg carbon or more (Janzen, 2006); conversely land-use or management change can offer an opportunity for sequestering atmospheric carbon in soils (Janzen, 2006). Importantly, in the long term, these soil carbon changes can be greater than any above-ground carbon gains. Therefore, soils hold a key role in reducing atmospheric CO_2 levels and their management is subject to scientific (e.g. climate change scenarios) and political (e.g. Kyoto Protocol) analysis. Moreover, peatlands and other organic soils of cold and temperate regions are presently assumed to be a net sink of carbon but they might become a net carbon source (CO_2 and CH_4) with predicted increase in global temperatures (Lal, 2004; Walter *et al.*, 2006). Bellamy *et al.* (2005) and other authors suggest this is already happening.

As even small changes in soil organic carbon pools, due to climatic changes or to human activities, might have large impacts on the global carbon cycle (Garten

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