Work package 2:

Ecosystem CO2 flux components and their interaction with the nitrogen cycle

Aims of WP2

* To quantify the contribution of the major ecosystem components to NEE
* To analyse and interpret their response to nitrogen contents and environmental driving forces
* To provide estimates of off-site carbon losses

Deliverables

* Response curves of phytoelement (leaf, stem, litter) CO2 exchange rates to environmental driving forces and tissue nitrogen contents for selected key species/functional groups
* Continuous data sets for bulk soil respiration
* Partitioning of bulk soil respiration into root and microbial contributions
* Response curves of root and soil microbial respiration to temperature, water and nitrogen content
* Partitioning of the net ecosystem fluxes into its main components for several episodic field campaigns during the season
* Isotopic characterisation of key species
* Complete data sets for the isotopic signature of trace gases at selected days (spring, summer fall) during the vegetation period
* Amounts of off-site carbon losses
* Amounts of nitrogen input into the ecosystem

Summary of tasks

No.	Work description
1	The response of phytoelement (leaf, stem, litter) CO_2 exchange rates to photosynthetically active radiation, temperature, CO_2 concentration, air water vapour deficit, soil water availability and tissue nitrogen contents will be measured both in the field and laboratory by means of CO_2/H_2O-porometers for selected dominant species within different functional groups. Seasonal effects (phenology, water and cold stress) on physiology will be considered.
2	Bulk soil respiration will be measured episodically with respiration chambers in the field. Continuous data sets of soil respiration will be inferred from measured soil temperatures and the temperature dependency of soil respiration.
3	Root and microbial contributions to bulk soil respiration will be quantified by means of the substrate induced respiration method (SIR).
4	The dependency of the root and microbial soil respiration components on temperature, water and nitrogen content will be measured with oxygen electrodes or infra-red gas analysers.
5	Phytoelement and soil nitrogen contents will be measured by means of an elemental analyser.
6	Net flux partitioning into the canopy and soil component will be accomplished by the combination of flux measurements (WP 1 and this work package) and the analysis of the stable C and O isotopes. Two mass spectrometers are available and equipped with the periphery necessary for the analysis of organic, water and gaseous samples.
7	Off-site carbon losses via grazing and harvesting will be quantified combining harvesting (cf. WP 3) and literature data and will permit an assessment of net biome production (NBP = NEE – off-site carbon losses).
8	Nitrogen inputs into the ecosystem will be determined by assessing atmospheric nitrogen deposition, inorganic and manure inputs.

Detailed assessment of the contribution of each partner team to the work description above, separately for each of the 23 study sites:

	UIBK					UBT		UNITUS		CEA		CEH		ULUND		UHEL		CEAM		LAC		SIRG	
	A1	A2	A3	A4	A5	G1	G2	I1	I2	I3	I4	UK1	UK2	S1	S2	F1	F2	E1	E2	CH1	CH2	CH1	CH2
1	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	-	-	v	v
2	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	-	-
3	10	10	10	10	10	10	10	-	-	10	10	-	-	-	-	-	-	v	v	-	-	v	v
4	v	v	v	v	v	v	v	v	v	-	-	v	v	v	v	v	v	v	v	v	v	-	-
5	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	-	-	v	v
6	10	10	10	10	10	10	10	-	-	10	10	-	-	-	-	-	-	-	-	-	-	v	v
7	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	-	-
8	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	-	-

Task 1 Phytoelement (leaf, stem, litter) CO_2 exchange rates

The response of phytoelement (leaf, stem, litter) CO_2 exchange rates to photosynthetically active radiation, temperature, CO_2 concentration, air water vapour deficit, soil water availability and tissue nitrogen contents will be measured both in the field and laboratory by means of CO_2/H_2O-porometers for selected dominant species within different functional groups. Seasonal effects (phenology, water and cold stress) on physiology will be considered.

Response curves for leaves and stems should be taken on fully developed, mature plant material during the period of maximum canopy growth. For different functional groups (graminoids, non-leguminous and leguminous forbs, dwarf shrubs, shrubs; at the Nordic sites lichens and mosses) the most dominant species should be selected (i.e. species contributing the largest percentage to canopy leaf area index and above-ground biomass). Response curves should cover the range of environmental conditions encountered in the field and should be suitable for parameterising the biochemical model of CO2 assimilation by Farquhar and co-workers (Farquhar 1979, Farquhar et al. 1980, Farquhar & von Caemmerer 1982), as modified by Harley and Tenhunen (1991) and Wohlfahrt et al (1998), or any other leaf model that is required for running an ecosystem gas exchange model as defined in work package 4. Minimum requirements for estimating parameters for the modified Farqhuar model are:

1.) the nitrogen dependency of light-saturated photosynthesis at ambient CO_2, average ambient humidity and a reference temperature (20° C), at least 15 replicates per species. *Note: The range of leaf nitrogen concentrations for this number of replicates is expected to provide a sufficient spread for deriving nitrogen dependencies. In addition, a portable chlorophyll metre (e.g. SPAD) can provide an in situ estimate of relative N concentrations.*

2.) the temperature response of light-saturated photosynthesis at ambient CO_2, average ambient humidity, covering the range of leaf temperatures occurring at the sites, and the highest temperature exceeding optimum temperature by at least 5° C, at least 8 replicates per species

3.) the nitrogen dependency of dark respiration at the reference temperature (20° C), on leaves that were kept in full darkness for at least two hours, at least 15 replicates per species. *Cf. Note for 1)*

4.) the temperature response of dark respiration, on leaves that were kept in full darkness for at least two hours, at least 8 replicates per species

All these measurements should include careful assessment of stomatal conductance, so that minimum and maximum stomatal conductances can be inferred and stress effects e.g. due to water shortage or low temperatures can be detected.

Measurements should be taken during the period of maximum canopy growth. As far as possible, additional measurements should be after the beginning and towards the end of the vegetation period.

Note: The suggested approach assumes that $v_{C_{max}}$ and J_{max} can be scaled directly (Wullschleger, Leuning 1997, Wohlfahrt et al. 1999a), and that possible differences in the temperature dependence of these parameters will not affect canopy photosynthesis significantly (Wohlfahrt et al. 1999b).

As far as possible additional measurements should be carried out that allow to assess vc_{max} and J_{max} and their respective temperature response more precisely. For this purpose light-saturated CO_2 response curves should be obtained at 10° C steps, covering the range of leaf temperatures occurring at the sites, and the highest temperature exceeding optimum temperature by at least 5° C. The following sequence of CO_2 partial pressure is suggested: 35, 10, 20, 120. For each temperature step the CO_2 response of at least 3 leaves per species should be included.

At sites where a significant reduction of gas exchange due to air and soil humidity, and/ or frost, is likely to occur, additional measurements should be carried out that allow the establishment of an empirical relationship quantifying these stress effects on leaf conductance, photosynthesis and respiration. Water stress are expected to occur at the Mediterranean sites. At the Nordic sites, especially in the Scandes, effects of water table and thaw depth on gas exchange will be considered.

Leaf parameters measured (times per season)

	Response of A to					**Response of R to**		**Functional groups studied**
	N	**T**	**I**	**CO_2**	**Vpd**	**N**	**T**	
UIBK	3	3	1	1	-	3	3	G, FN, FL, DS
UBT	1	1		1		1	1	G, FN, FL, (DS)
UNITUS	3	3			3	3	3	G, FN, FL
CEA	1	1	1	1		1	1	G, FN, FL, DS
CEH	1	1				1	1	G, FN, DS, S, M
ULUND	3	3				3	3	G, FN, DS, M, L
UHEL		3	3		3	1	1	G, DS, M, L
CEAM	3	3			3	3	3	G, FN, FL, S
SIRG	3	3	1	1	-	3	3	G, FN, FL, DS

G … graminoids, FN … non-leguminous forbs, FL … leguminous forbs, DS … dwarf shrubs, S … shrubs, M … mosses, L … lichens

Stem respiration

At sites where non-green stems make up a high percentage of canopy biomass, their temperature response will need to be assessed. Temperature response should cover the range of temperatures occurring at the sites.

Phytoelement gas exchange – further analyses of samples

SLA will be measured on water-saturated leaves for all leaf samples *(for technical details refer to Garnier et al. 2001).*

Phytoelements will be oven-dried until weight-constancy at 70 ° C

Nitrogen concentrations will be measured for all samples (cf. task 5).

Task 2: Bulk soil respiration

Bulk soil respiration will be measured episodically with respiration chambers in the field. Continuous data sets of soil respiration will be inferred from measured soil temperatures and the temperature dependency of soil respiration.

We define **soil respiration as $R_S = R_A + R_H$**, where R_A is the root respiration, (also called autotrophic respiration) and R_H the respiration of the micro organisms (also called heterotrophic respiration).

Our project includes a large North South transect within which a considerable heterogeneity is found. We agreed during the kick off meeting in Innsbruck, Feb. 2002, to apply the most appropriate method (see below) for each ecosystem type (basically three methods, see below). More detailed discussions on the different soil respiration methods are in appendices A and B. Irrespective of the applied methods the soil temperatures and moisture in different depths must be measured, as close to the cuvettes as possible to correlate these parameters with the respiration rate. Place the temperature sensors within the cuvette (in 2cm, 5cm, and ev. 10cm soil depth), well before the measurements. The more voluminous soil moisture measuring devices should be placed outside the cuvette, as close as possible, without disturbing the measured soil. Depending on the spatial variation of soil respiration in the field, five to ten replicates should be measured for each ecosystem studied.

Measurement Methodologies

Open, dynamic system:

This method is mostly used for continuous measurements and monitoring.

The cuvette, a plastic or metal ring is 10cm to 20cm high and between 10cm and 30cm in diameter, covering a soil surface area from 80cm^2 to 710 cm^2. The ring is covered by a lid, which is equipped with three tube fittings. One fitting is connected to an air pump leading air from 200 cm above ground via a mass flow controller into the cuvette (Flow 1). The second fitting leads the air via pump from the soil respiration chamber to the IRGA (Flow 2). The third fitting is connected to a short tube leading to the free atmosphere, preventing pressure differences between the cuvette and the ambient air and avoiding changes in the diffusion processes (pressurizing the chamber leads to underestimation or elimination of the CO_2 flux, while a pressure below ambient leads to overestimated respiration rates).

Tubing to the IRGA and pumps:
Important place a **filter** BEFORE the IRGA inlet avoiding pollution of the cuvette with dust and rub off material form the pump. Filter pore width of max. 5µm or less are recommended. Filters provided by LiCor proved to be very good. Although IRGA's of the newer generation are equipped with pressure gauges compensating automatically for pressure changes, it is still safest to place the pump before the IRGA to avoid pressure changes within the IRGA, which can cause false measurements. If possible use metal membrane pumps. Most rubber, perbunan or teflon membranes slowly deteriorate and release material particles and even CO_2, impairing the CO_2 measurements or polluting the IRGA.
If using **absolute IRGA**, the CO_2 concentration of the air sample before and after the soil respiration cuvette is measured. The respiration rate is calculated from the difference between the CO_2 concentration and the flow rate leading through the chamber (cf. appendix A).
If a **differential IRGA** is applied, a constant air flow form the air before the Cuvette led to the reference gas of the IRGA (placing the filters and pumps as mentioned above) and the sample gas after the chamber to the sample port of the IRGA. The calculation is done as above. For more details and recommendations see appendix A.

Closed, dynamic system

This type of method is mostly applied for **episodic measurements** and allows a **great number of sampling.** The installation of the chamber is done the same way, as described above. The chamber is connected to the CO_2 measuring device with two tubes. In this closed system one tube leads the measured air from the analysis system (containing a pump, mass flow controller, IRGA, data logger and controller) to the respiration chamber, while the other tube leads the air from the cuvette back to the analysis system where its change in CO_2 concentration is measured. Due to soil respiration the CO_2 concentration increases in the chamber within a certain time. Either **a)** the increase of the CO_2 concentration is measured within a preset time or **b)** the time is measured within which a preset increase in the CO_2 concentration has been reached. The latter is recommended for soils with a low respiration rate for improved precision (See recommendations appendix A).

Laboratory measurements:

Laboratory measurements should only be applied in addition to *in situ* measurements to obtain an improved characterization of soils (cf. task 4). Soil cores of different sizes are extracted form the soil. These samples are stored in the laboratory under a given temperature and air humidity. In some cases the soil is processed (eg. removal of the plant material, and sieving of the soil etc.) and incubated. The CO_2 released from such soil samples is usually analyzed with a gas chromatograph or an IRGA, using small samples of gas.

For further details and recommendations on soil respiration measurements please refer to the supporting documents.

Task 3: Partitioning of soil respiration

Root and microbial contributions to bulk soil respiration will be quantified by means of the **s**ubstrate **i**nduced **r**espiration method (SIR).

The soil respiration will be partitioned into the two major components $\mathbf{R_A}$ and $\mathbf{R_H}$. Based on the fact that C3 plants discriminate the heavier ^{13}C against the lighter ^{12}C isotope during photosynthesis, plant material contains less ^{13}C (Delta^{13}C ˜ -27‰) than the ambient air (Delta^{13}C ˜ -8‰). Thus soil respiration releases a lighter CO_2 (- 25‰ and -23‰) than that of the air, since the respired substrates is plant derived material (organic matter and the sugar components, synthesized via photosynthesis). The **s**ubstrate **i**nduced **r**espiration method (SIR) makes use of the fact, that the two sources of respiratory CO_2 use different substrates, the micro-organisms the soil organic material and the roots the sugar produced in the leaves. Adding sugar form C4 plants to the soil (C4 plants do not discriminate the ^{13}C against the lighter ^{12}C isotope during photosynthesis and therefore contain more ^{13}C (Delta^{13}C ˜ -12‰)), the microorganisms will immediately utilize the much easier available C4 sugar. Thus the Delta^{13}C released from the microorganisms is much more positive (Delta^{13}C ˜ -12‰) than that released by the roots. The total CO_2 emerging from the soil is a mixture of microorganism and root respiratory CO_2. The Delta^{13}C value of the total soil respiration will be enriched in ^{13}C compared to the controls, depending on the proportion of root / microorganism respiration. This mixing ratio between the Delta^{13}C of the total respiration before and the application of the C4 sugar and the Delta^{13}C values of the organic root material allow to calculate the proportion of the root and that of the microorganism respiration. (A detailed description is given in Ekblad and Högberg; 2000).

Method: Respiration chambers are installed the same way as described for bulk soil respiration. In one set of cuvettes an amount of 500ml – 1500ml of C4-sugar water is injected into the soil, while the same amount of pure water is applied to the control set of cuvettes. In 2 hourly intervals the cuvettes are covered with a lid for ca. 5 Minutes. Thus the respiration rate can be measured. Before removing the lid, cuvette air is sampled for the isotopic analysis with a syringe. This procedure is repeated until a plateau in Delta^{13}C is reached. This maximum value is then used for the calculation. For each soil type preliminary tests must be performed to get an estimate for the time needed until a Delta^{13}C maximum is reached.

Before the CO_2 sampling glass tubes are filled with pure nitrogen and sealed with a membrane cap. The gas samples are injected into these glass tubes with a 13ml syringe, while a second needle punctures the membrane to release the pressure built up in the tube, during sample injection. The glass tubes (vacutainers) must be transferred to the lab immediately. The samples are analyzed with an isotope ratio mass spectrometer connected to a gas bench for the purification of the air samples. More information will be given before the sampling takes place on the sites.

This task is done by the SIRG Group on selected sites only due to limited capacity.

Task 4: Root and microbial respiration

The dependency of the root respiration on temperature, water and nitrogen content will be measured with oxygen electrodes or infra-red gas analysers. Roots of the main rooting horizon will be extracted with soil cores. Roots should be freed from soil by washing with deionised water. Root respiration should be measured within 5 hours after extraction. Roots should be kept moist throughout the treatment. Roots should be injured as little as possible. Dead roots, as far as detectable by their darker colour and brittleness, should be removed prior to measurement of root samples. Fine (< 2mm) and coarse roots (> 2 mm) should be measured separately. Root respiration will be measured using an oxygen electrode or an IRGA. Using an IRGA, root respiration can be measured at ambient CO2-concentration or at CO2 concentrations similar to those experienced by roots. In the latter case, great care should be taken to avoid leakages of the system, which would result in an underestimation of root respiration. Temperature response of root respiration should cover the range of soil temperatures occurring in the field. After respiration measurements roots should be oven-dried at 70° C and subsequently analysed for N-concentrations.

To assess the contribution of root respiration to bulk soil respiration it will be essential to analyse the amount of fine and coarse roots present per m² soil surface, as well as their nitrogen concentrations (WP 3).

Heterotrophic respiration will be estimated by subtracting root respiration per m² soil surface from bulk soil respiration.

Microbial respiration and microbial biomass: For a further characterisation of soils basal respiration is measured in moist samples (approx. 60% WHC) of sieved (2mm) soil. Approximately 100 g of soil are needed. The CO_2 evolution rate is recorded hourly using an automated infrared (IR) gas analyzer system for 18 hours. The CO_2 output of the last 3 h is usually taken as basal respiration (after a sample has reached a constant CO_2 evolution rate). After basal respiration measurement, the samples are amended with 1% glucose, and then the substrate induced respiration (SIR) is measured, which can be directly converted to microbial biomass (Anderson & Domsch, 1978). All measurements are performed at 22°C ± 0.5. Finally, from respiration and microbial biomass, the metabolic quotient (qCO_2), an important ecophysiological indicator, is calculated.

Task 5: Phytoelement and soil nitrogen contents

Phytoelement and soil nitrogen contents will be measured by means of an elemental analyser or Kjeldahl technique (cf. Table 2). Cross-calibrations of analysers will be achieved following a protocol to be provided by applying the same standards. Plant material will be dried at 70 °C until weight-constancy.

Task 6: Net flux partitioning into the canopy and soil component

Net flux partitioning into the canopy and soil component will be accomplished by the combination of flux measurements (WP 1 and this work package) and the analysis of the stable C and O isotopes. Two mass spectrometers are available and equipped with the periphery necessary for the analysis of organic, water and gaseous samples.

As outlined in Task 3, the Delta^{13}C released form the soil differs form that of the air. Based on these differences the respiratory flux component can be separated from the C-assimilation during the day. At night the Delta^{13}C of the CO_2 released by the above ground plant components is very similar to the Delta^{13}C of the soil respiratory CO_2. For the separation of the above and below ground respiratory proportions the CO_2 is therefore analyzed for the ^{18}O / ^{16}O isotope ratio. Due to transpiration the leaf water is enriched in ^{18}O relative to the soil water. CO_2 dissociates in water, thus the oxygen atoms can exchange between the water and CO_2 molecules, which is enhanced by the carboanhydrase enzyme. Thus the Delta^{18}O of the CO_2 released from the soil is distinctly different form that of the soil.

Method: With a specially designed gas sampling device glass flasks are filled in a diurnal course with air 200cm above, within the canopy and directly above the soil. The samples are analyzed with an isotope ratio mass spectrometer connected to a gas bench for the purification of the air samples. More information will be given before the sampling takes place on the sites.

This task is done by the SIRG Group on selected sites only due to limited capacity.

Task 7: Off-site carbon losses

Off-site carbon losses via grazing and harvesting will be quantified combining harvesting (cf. WP 3) and literature data and will permit an assessment of net biome production (NBP = NEE – off-site carbon losses).

Task 8: Nitrogen inputs into the ecosystem

Nitrogen inputs into the ecosystem will be determined by assessing atmospheric nitrogen deposition, inorganic and manure inputs.

Atmospheric deposition will be assessed by two approximations:

1. a combination of bulk deposition measurements and branch washing
2. air concentration measurements applying passive samplers and denuders

Approximation 1:

Bulk deposition: To collect rain for chemical analysis, a 10 l bulk collector composed of a polypropylene bucket connected by a U-bend, to prevent evaporation, to a funnel is recommended. The rim of the funnel has to be positioned at 1.5 m above ground level to avoid ground contamination. If possible an external ring to avoid bird problems should be installed. Before collecting the sample, the funnel should be rinsed with deionized water to wash away any matter deposited between the last rain episode and the collection time. Thus, such samples represent a combination of dry + wet deposition, with the dry component being mostly coarse particulate matter (Krupa, 2000). The sampling period has to be not longer than two weeks (14 days), pereferably one week. Once in the lab, pH, conductivity and alcalinity have to measured for each sample. At least 4 collectors are recommended per site.

Branch washing: Because of the inadequacy of any single method to evaluate dry deposition, we recommend to measure deposition to branchlets of abundant shrub or tree species nearby the grassland studied, to be compared with the bulk deposition data. Four open exposed individual trees or shrub (all form same species) present near the tower or measurement area, four branches (5-8 cm long), one from each geographic quadrant, at about 1-1.5 m above ground level, should be thoroughly washed at the beginning of the experiment and rinsed every 2 weeks with 50 ml of deionized-distilled water dispersed from polypropylene spray bottles. The rinsing solutions should be collected into a funnel in polyethylene bottles and immediately placed in cool boxes at 4º C. Once in the lab a composed sample of 200 ml should be obtained for each tree, and the same analytical procedure as for bulk deposition samples should be followed. Total surface area exposed should be assessed.

Approximation 2:

Air concentrations –Passive Samplers: NO_2 (even Nox) and NH_3 air concentrations should be measured continuously by passive samplers (one or two weeks average, depending on the site). Commercial devices are available, if partners use different devices intercomparations should be carried out. HNO_3 passive samplers can be used, from CEAM (under development, experimental ones). Nitrate from nylon filters, a product of HNO3 absorption, was also extracted with nanopure water, and its concentrations can be determined colorimetrically (TRAACS 2000 Bran & Lueble Instrument) or by IC. HNO3 samplers can be calibrated against honeycomb denuder systems in continuously stirred tank reactor (CSTR) chambers. All passive samplers have to be placed about 2 m above the ground on poles — each sampler contained two to three replicate filters subsequently used for analyses.

Air concentrations –Dennuders/Filter-pack: A series of dennuders combined with filter-packs is recommended in a field campaign mode (seasonally, 4 days per season) to collect particles (<2.5 um) and gases simultaneously.

For further details and recommendations please refer to the supporting document.

Table 2: Output parameters

Parameter	Unit
Photosynthesis (A)	µmol m^{-2} s^{-1}
Leaf Respiration (R_{leaf})	µmol m^{-2} s^{-1}
Bulk Soil Respiration (R_{soil})	µmol m^{-2} s^{-1}
Root Respiration (R_{root})	nmol g^{-1} s^{-1}
Nitrogen Concentration (N)	mmol g^{-1}
N-deposition	g N m^{-2} a^{-1}
Off-site C-loss	g C m^{-2} a^{-1}
Isotope ratio from respiratory soil CO_2	Delta^{13}C $_{vs\ PDB}$ [‰]
Isotope ratio from canopy & soil gas exchange (CO_2)	Delta^{13}C $_{vs\ PDB}$ [‰], Delta^{18}O $_{vs\ VSMOW}$ [‰]

Table 3: Instrumentation used

	Phytoelement gas exchange	**Bulk soil respiration**	**Nitrogen contents**
UIBK	CIRAS	O (c), C (e)	EA
UBT	Li-6400	C (e)	EA
UNITUS	ADC	O (e), C (e)	EA
CEA	Li-6400, (LiCOR fluorimeter)	C (e)	EA
CEH	Li-6400	C (e)	K
ULUND	Li-6400, CIRAS	C (+ new system)	EA/ K
UHEL	CIRAS	C	EA
CEAM	Walz, ADC	C (c, e)	K
SIRG	Li-6400	C (e)	EA

O ... open, C ... closed system, c ... continuous, e ... episodic

EA ... elemental analyser, K ... Kjeldahl