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# Ecological metabolomics: overview of current developments and future challenges

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**Abstract** Ecometabolomics, which aims to analyze the metabolome, the total number of metabolites and its shifts in response to environmental changes, is gaining importance in ecological studies because of the increasing use of new technical advances, such as modern HNMR spectrometers and GC-MS coupled to bioinformatic advances. We review here the state of the art and the perspectives of ecometabolomics. The studies available demonstrate ecometabolomic techniques have great sensitivity in detecting the phenotypic mechanisms and key molecules underlying organism responses to abiotic environmental changes to biotic interactions. But such studies are still scarce, and in most cases they are limited to the direct effects of a single abiotic factor or of biotic interactions between two trophic levels under controlled conditions. Several exciting challenges remain to be achieved through the use of ecometabolomics in field conditions, involving more than two trophic levels, or combining the effects of abiotic gradients with intra- and inter-specific relationships. The coupling of ecometabolomic studies with genomics, transcriptomics, ecosystem stoichiometry, community biology and biogeochemistry may provide a further step forward in many areas of ecological sciences, including stress responses, species lifestyle, life history variation, population structure, trophic interaction, nutrient cycling, ecological niche and global change.

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## Introduction

The possibility of using progressively improved metabolomic techniques in ecophysiological and ecological studies has opened up a new way to advance knowledge of the structure and function of organisms and ecosystems. Metabolomics is the analysis of the complete metabolome (all the metabolites that one organism produces) at one moment (Fiehn 2002). It provides the phenotypical response at the metabolic level in a particular environmental circumstance. Moreover, it is also a powerful tool to monitor the phenotypic variability of one genotype in response to environmental changes in drought (Fumagalli et al. 2009), nutrient availability (Hirai et al. 2004, 2005), pollutants (Jones et al. 2007; Bundy et al. 2008), salinity (Fugamalli et al. 2009), temperature (Michaud and Delinger 2007) and biotic interactions (Choi et al. 2006), among other ecological factors. These studies are especially adequate in plants because metabolomic studies enable the simultaneous analysis of primary compounds together with secondary compounds, which have a defensive and protective function.

Metabolomics provides a better analysis of the different response capacities conferred by the phenotypic plasticity of each species, allowing to ascertain what metabolic pathways are involved in a phenotypic response. Moreover, this facilitates transcriptomics change research (Hirai et al.

2004; Brosché et al. 2005; Fukushima et al. 2009a). Metabolomics can also be coupled to genomic studies for faster determination of the genes involved in adaptive responses (Bino et al. 2004; Oksman-Caldentey and Saito 2005). This approach therefore has great potential to elucidate gene function and to establish data networks. In this way, it advances our knowledge of the development of phenotypic plasticity (Via et al. 1995; Pigliucci 2005) and its evolutionary consequences (Agrawal 2001). In this regard, metabolomics has several advantages compared to more conventional methods of analyses in chemical ecology (extract fractionation, purification and bioassays). Since all compounds are measured at once in only one step, rather than put through iterative purification steps, unstable compounds are more likely to be detected and measured. Metabolomics can also be used as a preliminary screening study of the metabolome response. This does not exclude the simultaneous or subsequent use of target chemical analyses.

Recent rapid improvements in analytical methods and in the ability of computer hardware and software to interpret and visualize large data sets (Gehlenborg et al. 2010) have multiplied the possibilities of rapidly identifying and simultaneously quantifying an increasing number of compounds (e.g., carbohydrates, amino acids and peptides, lipids, phenolics and terpenoids). These advances will enable us not only to take 'static pictures' or snapshots of the metabolome, but also to capture and to 'film' its dynamic nature. Ecological metabolomics can thus serve as a powerful indication for defining organism lifestyle. All in all, we may now be able to achieve a dynamic, holistic view of the metabolism and health of an organism, a population or an ecosystem, and in this fashion open the door to exciting new insights in ecology.

This study reviews the state of the art of ecological applications of metabolomic techniques, giving an overview of the current findings reached by its use in ecophysiological and ecological studies. We also discuss the possible future contribution of metabolomics to progress in ecophysiology and ecology. To achieve such progress, we highlight the need to couple metabolomic studies with other omic studies such as genomics, transcriptomics and proteomics. The aim is to reach an overview of organism response to environmental changes at different time scales and from genotype to phenotype. We also discuss the ecological topics where metabolomics could be more successfully used. Among these topics we highlight ecosystem stoichiometry, plant-herbivore-predator systems, parasitism, climate change and invasive species, among others (Fig. 1).

## Ecometabolomics

We use here terminology based on that of Fiehn (2002) and Schripsema (2010), but simplified for metabolomic techniques applied to ecological studies. Briefly, the study type is called metabolomic profiling when the aim is the quantitative analysis of a set of metabolites of a selected number of metabolic pathways or of metabolite types (e.g., polar, semipolar or non-polar) with or without its identification. We propose to call partial ecometabolomic studies (PEM)

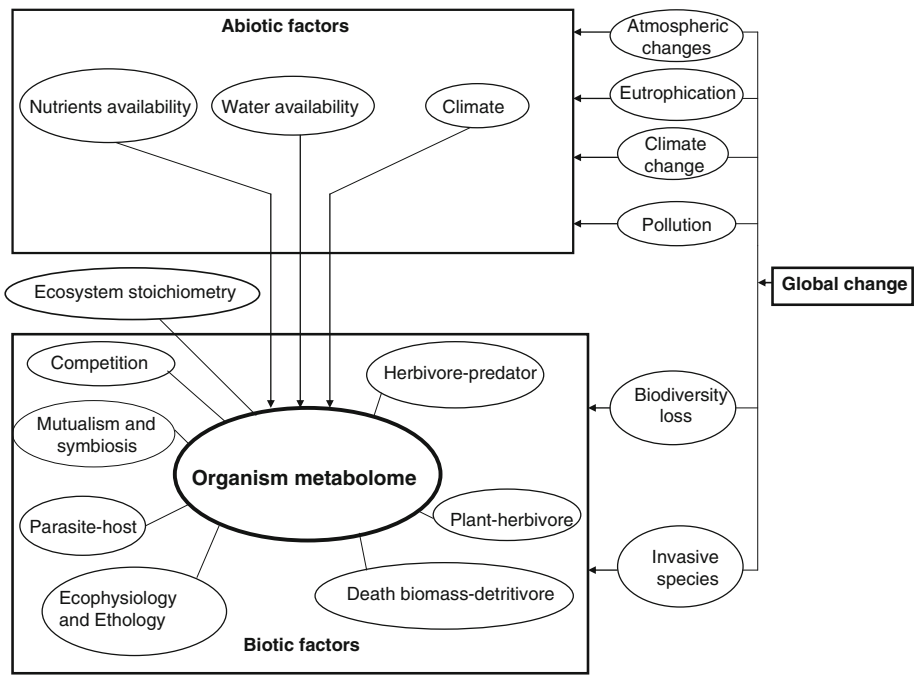


**Fig. 1** Ecological topics where ecometabolomics should represent a direct tool to advance

those metobolomic profiling studies applied to ecophysiological or ecological studies that aim to elucidate the effects of biotic or abiotic factors on a specific whole pathway or intersecting pathways by identifying the metabolite or set of metabolites involved in organism response to environmental changes by using general qualitative and quantitative analytical techniques such as NMR or GC-MS and data-mining statistical analyses. In this way the target studies using more target techniques such as HPLC focused on the study of some limited set of metabolites are not considered ecometabolomic studies.

When it is not required or possible to identify every metabolite, it is often sufficient to rapidly classify samples according to their origin or their ecological or ecophysiological relevance. This process is called metabolic fingerprinting, which now in an ecological context we propose to name ecometabolic fingerprinting. This "holistic" method enables unbiased exploration and examination of sample molecular biochemistry and through suitable interpretation can be used to study plant responses to environmental changes (Gidman et al. 2005, 2006). When the aim is to obtain information about the whole metabolome (the total number of metabolites in one biological system) by identifying and quantifying as many metabolites as possible, we must conduct an analysis approach such as NMR or GC-MS that enables determining and quantifying the maximum number of metabolites. Since such an approach reveals the maximum information about the metabolome of the biological system under study as possible, this approach is called metabolomics. In an ecological context, when the objective is to discern the global metabolomic response of an organism to environmental changes, we propose to call it ecometabolomics. In fact, this latter approach, ecometabolomics, is the most appropriate to comprehend the complete response of an organism to environmental changes.

## Analytical techniques

In metabolomic studies it is very important to take into account the pre-analysis treatment. To prevent post-sampling hydrolysis of some compounds, it is important to immediately freeze the sample (most frequently by introducing it immediately into $N_2$ liquid) and thereafter to lyophilize it to completely dry it until the extraction process (see Kim and Verpoorte 2010 for more details on sample preparation). Since all metabolites can provide information about species' responses to environmental changes, ecometabolomic studies should be designed to detect as many metabolites as possible. As no single solvent allows all metabolites to be extracted, combinations of several different solvents can be used. Kim and Verpoorte have tested different extraction methods for NMR plant metabolomics. The use of a two-phase solvent system, composed of a mixture of chloroform, methanol and water (2:1:1, v/v), has proven to be the most advisable method (Choi et al. 2004). For detailed information about the different extraction properties, see Kim and Verpoorte (2010) and Kaiser et al. (2009).

Currently no single analytical method or combination of methods (i.e., chromatography combined with mass spectroscopy) can detect all metabolites (estimated to be between 100,000 and 200,000 in the plant kingdom) within a given biological sample. Gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS) and nuclear magnetic resonance spectrometry (NMR) are the procedures with the best capacity to determine the widest ranging sets of metabolites. GC-MS has proven to be a robust tool for the study of volatile organic compounds (Degen et al. 2004; Ozawa et al. 2008; Llusia et al. 2010), but GC-MS analysis of extracts containing other analytes such as organic acids, sugars, amino acids and steroids is complicated. Many metabolites are non-volatile and must be derivatized prior to GC-MS analysis (Gullberg et al. 2004). In such cases, thermolabile compounds may be lost. Moreover, it is difficult to elucidate the unknown structures of metabolites by using GC-MS alone. LC-MS is of particular importance to study a great number of metabolic pathways at once since plant metabolism embodies a huge range of semi-polar compounds, including many key groups of secondary metabolites, which are better separated and detected by LC-MS (Allwood and Goodacre 2009). Thus, while GC-MS is best suited for compound classes appearing mainly in primary metabolism (frequently after derivation), i.e., amino acids, fatty acids and sugars or volatile compounds, LC-MS is more adequate to determine the overall biochemical richness of plants including several semi-polar groups of secondary metabolites. To gain structural elucidation power, the method of collision-induced dissociation can be used (Jennings 2000). The parent ions providing electrospray ionization (ESI) are isolated and accelerated in mass spectrometry using mass filters, so as to collide with molecules of bath gas, giving rise to the fragment spectrum (MS/MS method). Fourier transform-ion cyclotron resonance mass spectrometry (FT-ICR-MS) and ultra-pressure liquid chromatography mass spectrometry (UHPLC-MS) can be used to further increase the number of detectable metabolites. FT-ICR-MS is a very high-resolution technique in that masses can be determined with very high accuracy. This is due to the great sensitivity of this method to separate compounds with different mass-to-charge ratios ($m/z$) by their different cyclotron frequency in a fixed magnetic field. This method together with previous chromatographic methods has been scarcely used in

ecometabolomic studies until now (Hirai et al. 2004; Haesler et al. 2008), but the results are promising. UHPLC-MS constitutes an improvement in the power of separating compounds during the chromatographic phase (high-resolution capacity) with respect to the conventional HPLC-MS method. This is achieved by the application of great pressure to the carrier solution that reduces the dispersion of each chemical compound in the separation column. Moreover, UHPLC permits shortening the time of the separation phase, a fact especially interesting in ecometabolic studies where great number of samples must be processed.

$^{1}$H-NMR has proven to be an appropriate tool for untarget analyses. It has the advantage that it can be applied to determine polar, semi-polar and non-polar metabolites, and that it produces signals that directly and linearly correlated with compound abundance (Lewis et al. 2007). However, NMR spectroscopy has intrinsic low sensitivity for low concentrations of metabolites and signal overlapping for complex mixtures. This can at times be problematic for structural elucidation of a metabolite at low concentrations. The use of low temperatures to stabilize the detector by modern cryogenically cooled devices can improve the sensitivity up to a factor of five by reducing the thermal noise from the electronics of the NMR spectrometer. Two-dimensional (2D) NMR spectroscopy methods and high-resolution magic angle spinning (Sekiyama et al. 2010) further improve the sensitivity. 2D NMR spectroscopy provides an increased signal dispersion, thus enabling the detection of connectivity between signals and hence helping to identify metabolites. This method includes the total angular momentum (J) resolved method ($^{1}$H-$^{1}$H 2D J resolved), correlation spectroscopy (COSY) and total correlation spectroscopy (TOCSY). $^{1}$H-$^{1}$H 2D J resolved yields information on the multiplicity and coupling patterns of resonances, which reduces the degree of spectra complexity but retains all the chemical shifts and the relative intensity of spectral peaks. COSY and TCOSY provide $^{1}$H-$^{1}$H spin-spin coupling connectivities, providing information as to which hydrogens in a molecule are closer in terms of chemical bonds. In the high-resolution magic angle spinning approach, $^{13}$C is detected indirectly using the more abundant $^{1}$H by using spin-spin interaction $^{13}$C-$^{1}$H. This yields both coordinated $^{13}$C-$^{1}$H-NMR shifts that are useful for identification purposes. Moreover, the stable isotopes $^{13}$C, $^{15}$N and $^{31}$P have been successfully employed in NMR in vivo metabolomic studies in ecophysiological studies (Lundberg and Lundquist 2004; Kikuchi et al. 2004). When for the aim of a study it is important to elucidate chemical structures that probably are unknown, such as for example in plant herbivore relationships, NMR-based metabolomic studies are the most adequate tool (see a recent review on this topic by Leiss et al. 2011). For more detailed information on the advantages and disadvantages of each method, see Summer et al. (2003), Kopka et al. (2004), Moco et al. (2007) and Verpoorte et al. (2008).

Recently, HPLC separation, diode array detection (DAD), MS detection, solid phase extraction (SPE) for enrichment of a metabolite and NMR, namely HPLC-DAD-MS-SPE-NMR (Tang et al. 2009), have been combined, enabling good separation, sensitivity and molecular structure elucidation all at once (Tang et al. 2009). For a further description of data acquisition methods in metabolomics, see Hall (2006), Allwood and Goodacre (2009) and Lindon and Nicholson (2008).

## Ecometabolic responses to abiotic factors

Several studies have investigated the responses of some metabolic pathways in organisms to changes in abiotic factors such as climate (temperature and water availability), nutrient availability, salinity or pollution (Tables 1, 2). Changes in the composition of some metabolite groups have been described using analytical target methods in response to changes in several environmental factors, such as drought (Llusia and Peñuelas et al. 1999; Llusia et al. 2008; Peñuelas et al. 2009), temperature (Peñuelas and Llusia 1999; Filella et al. 2007), pollutants (Peñuelas et al. 1999), irradiance (Peñuelas and Llusia 1999) or $CO_2$ (Peñuelas and Llusia 1997). Moreover, several studies have reported that the metabolites produced in response to abiotic or biotic environmental changes further interact with other abiotic and/or biotic ecosystem constituents, e.g., terpene emissions that affect the climatic and atmospheric conditions (Andreae and Crutzen 1997; Kavouras et al. 1998; Peñuelas and Llusia 2003; Peñuelas et al. 2009a; Peñuelas and Staudt 2010). Now ecometabolomic studies provide the possibility to take a step forward in knowledge at the level of global organism responses to environmental changes. Some reports have already begun to explore the possibilities of the use of metabolomic approaches in ecological studies.

### Climatic factors

Most work conducted in plants using partial ecometabolomic techniques to investigate metabolic changes under cold stress has involved analyzing the polar metabolites in *Arabidopsis* sp. grown in controlled conditions (Kaplan et al. 2004, 2007; Cook et al. 2004; Gray and Heath 2005; Davey et al. 2009; Maruyama et al. 2009; Korn et al. 2010), although other plant species have also been studied (Janda et al. 2007) (Table 1). The main responses to low temperatures included increases in metabolites related to amino acid-protein and soluble carbohydrates recognized

**Table 1** Ecometabolomic studies that have focused on abiotic effects on organism metabolism

| Species | Analytical techniques, study type | Main results | Reference |
|---|---|---|---|
| Cold | | | |
| *Dendrobaena* spp. | $^{1}$H NMR, PEM polar metabolites | ↑ Glucose | Bundy et al. (2003) |
| *Aporrectodea* spp. | | | |
| *Arabidopsis* sp. | GC-MS, PEM polar metabolites | ↑ Several amino acids, glucose, fructose, galactinol | Cook et al. (2004) |
| *Paspalum vaginatum* | GC-MS, PEM lipids | ↑ Linoleic acid (triunsaturated), fatty acids | Cyril et al. (2002) |
| *Arabidopsis lyrata* | MS, MF | Sugar was the main discriminating metabolite group | Davey et al. (2009) |
| *Arabidopsis* spp. | HPLC-MS, MF | Changes in metabolome fingerprinting | Gray and Heath (2005) |
| *Triticum aesticum* | GC-MS, PEM lipids | ↑ Fatty acids, unsaturated | Janda et al. (2007) |
| *Arabidopsis thaliana* | GC-MS, PEM polar metabolites | ↑ Several amino acids and sugars, phosphoric acid<br>↓ xylitol, mannitol | Kaplan et al. (2004, 2007) |
| *Arabidopsis thaliana* | GC-MS, PEM polar metabolites | ↑ Raffinose, glucose, galactose, sucrose, proline, glycine, maltitol, fumaric acid, succinic acid, galactinol, itaconic acid, ethanolamine | Korn et al. (2010) |
| *Beta vulgaris* | Thin layer chromatography, PEM lipids | ↑ Fatty acids, unsaturated | Lindberg et al. (2005) |
| *Arabidopsis* sp. | $^{1}$H NMR, HPLC-UV, PEM polar metabolites | ↑ Tetrahalose, maltose, alanine, sucrose, glutamine | Lugan et al. (2009) |
| *Arabidopsis thaliana* | GC-MS, HPLC-IT-MS, PEM polar metabolites | ↑ Starch degrading pathways, sugar alcohol synthesis, galactinol, raffinose, kaempfenol, 7-rhammoside | Maruyama et al. (2009) |
| *Sarcophaga crassipolpis* | **GC-MS and $^{1}$H NMR, polar and non-polar metabolites** | ↑ Urea, sorbitol, glutamine<br>↓ $\beta$-alanine, ornithine, trehalose | Michaud and Denlinger (2007) |
| *Belgica antarctica* | GC-MS, PEM polar metabolites | ↓ Serine | Michaud et al. (2008) |
| *Drosophila melanogaster* | $^{1}$H NMR, PEM polar and semipolar metabolites | ↑ Sugars | Overgaard et al. (2007) |
| Warming | | | |
| Different soils, soil metabolome (both of bulk soil and of microbes) | GC-MS, PEM polar and semipolar metabolites | ↑ Acetic acid, furanacetic acid, xylulose, phosphoric acid (in bulk soil)<br>↓ Galactonic acid, turanose (in bulk soil)<br>↑ Myristic acid, glutamic acid, thymidine, proline (in microbes)<br>↓ Thymidine (in microbes)<br>The metabolome of bulk soil was more sensitive to temperature than those of microbes when comparing different soils | Coucheney et al. (2008) |
| *Arabidopsis thaliana* | GC-MS, PEM polar metabolites | ↑ Several sugars, leucine, valine, tyrosine, uracil, quinic acid, xylitol | Kaplan et al. (2004) |
| *Agrostis stolonifera* | GC-MS, PEM lipids | ↑ Lipid, unsaturated | Larkindale and Huang (2004) |
| *Drosophila* spp. | $^{1}$H NMR, PEM polar metabolites | ↑ Leucine, valine, tyrosine | Malmendal et al. (2006) |
| *Belgica antarctica* | GC-MS, PEM polar metabolites | ↓ Serine | Michaud et al. (2008) |

**Table 1** continued

| Species | Analytical techniques, study type | Main results | Reference |
|---|---|---|---|
| *Schizosaccharomyces pombe* | LS-MS, PEM polar and semi-polar metabolites | ↑ Some amino acids, trehalose, glycerophosphoethanolamine, arabitol, ribulose, ophthalmic acid | Pluskal et al. (2010) |
| | | Many changes in secondary metabolites such as ↓ urea cycle intermediates and ↑ acetylated compounds | |
| *Arabidopsis* spp. | GC-MS, PEM polar metabolites | ↑ Sucrose, maltose, glucose | Rizhsky et al. (2004) |
| *Oncorhynchus mykiss* | $^{1}$H NMR, MF | Different metabolomic fingerprinting | Turner et al. (2007) |
| *Oncorhynchus mykiss* | $^{1}$H NMR, PEM polar metabolites | ↑ Antithermal stress protein pathways | Viant et al. (2003) |
| | | ↓ ATP, glycogen | |
| *Folsomia candida* | $^{1}$H NMR, PEM polar metabolites | ↓ Arginine, lysine, leucine, phenylalanine, tyrosine (after 7 h heat exposure) | Waagner et al. (2010) |
| *Oryza sativa* | Capillary electrophoresis-MS, PEM polar metabolites | ↑ Sucrose, pyruvate/oxalacetate-derived amino acids | Yamakawa and Hakata (2010) |
| | | ↓ Sugar phosphates and organic acids involved in glycolysis/gluconeogenesis and the tricarboxylic acid cycle (TCA) | |
| Drought | | | |
| *Zea mays* | HPLC-MS/MS, PEM polar metabolites | ↑ Threonine, GABA, 6-benzylaminopurine, proline, tryptophan, leucine | Alvarez et al. (2008) |
| *Pisum sativum* | $^{1}$H NMR, PEM polar metabolites | ↑ Proline, valine, threonine, homoserine, myoinositol, GABA | Charlton et al. (2008) |
| *Vitis vinifera* | GC-MS, PEM polar metabolites | ↑ Glucose, maltose, proline | Cramer et al. (2007) |
| *Lolium perenne* | GC-MS, PEM polar and semipolar metabolites | ↑ Glucose, raffinose, fructose, trehalose, maltose | Foito et al. (2009) |
| | | ↓ Fatty acids | |
| *Oryza sativa* | **$^{1}$H NMR, polar and non-polar metabolites** | ↑ Glucose, glutamate, glutamine | Fumagalli et al. (2009) |
| *Stagonosphera nodorum* | GC-MS, PEM polar and semipolar metabolites | ↑ Glycerol, arabitol | Lowe et al. (2008) |
| | | ↓ Several amino acids | |
| *Arabidopsis* sp. | $^{1}$H NMR, HPLC-UV, PEM polar metabolites | ↑ Proline, tyrosine, malate, GABA | Lugan et al. (2009) |
| *Belgica antartica* | GC-MS, PEM polar metabolites | ↑ Glycerol, arythritol | Michaud et al. (2008) |
| | | ↓ Serine | |
| *Lupinus albus* | $^{13}$C NMR, PEM polar metabolites | ↑ Sucrose, glucose, proline | Pinheiro et al. (2004) |
| *Arabidopsis* spp. | GC-MS, PEM polar metabolites | ↑ Sucrose, maltose, glucose, proline | Rizhsky et al. (2004) |
| *Solanum* sp. | GC-MS, PEM polar metabolites | ↑ Alanine, GABA, $\beta$-alanine, homoserine, isoleucine, proline, serine, valine | Semel et al. (2007) |
| | | ↓ Glutamine, glycine, cysteine | |
| Natural gradients | | | |

**Table 1** continued

| Species | Analytical techniques, study type | Main results | Reference |
|---|---|---|---|
| Differents climate and soil, *Pseudotsuga menxiesii* | GC-MS, PEM polar metabolites | Carbohydrate and lignin synthesis pathways were the most affected when comparing individuals of different sites | Robinson et al. (2007) |
| Different level of soil degradation. Soil worms | **$^1$H NMR, polar and non-polar metabolites** | Levels of glucose, maltose, alanine and triacylglycerides are potential biomarkers of worm stress due to soil quality decrease | Rochfort et al. (2009) |
| Different soils, *Populus tremuloides* (litter) | UPLC-MS, MF | Different metabolic fingerprinting in different soils | Wallenstein et al. (2010) |
| Nutrient stress | | | |
| N, P, S, Fe stress, *Chlamydomonas reinhardtii* | GC-MS, PEM polar metabolites | ↓ Some aa, soluble sugars, but different depending on the nutrient that induces the stress | Bölling and Fiehn (2005) |
| P stress, *Phaseolus vulgaris* (roots) | GC-MS, PEM polar metabolites | ↑ Polyols and sugars | Hernández et al. (2007) |
| N and P stress *Arabidopsis* spp | **HPLC-DAD, FT-MS, electrophoresis, polar and non-polar metabolites** | Changes in glucosinolate pathway | Hirai et al. (2004, 2005) |
| N and S stress, *Triticum aesticum* | $^1$H NMR, PEM amino acids | ↑ Glutamine | Howarth et al. (2008) |
| P stress, *Hordeum vulgare* | HPLC-MS, GC-MS, PEM polar and semipolar metabolites | ↑ Ammonium metabolism<br>↓ Sugar metabolism | Huang et al. (2008) |
| S stress, *Arabidopsis* spp. | HPLC-DAD, HPLC-MS, GC-MS, PEM polar metabolites | ↑ Purines, allantoine<br>↓ Several lipids | Nikiforova et al. (2005a) |
| N stress, *Solanum lycopersicum* | GC-MS, PEM polar metabolites | ↑ Several sugars and phosphoesters<br>↓ Several amino acids and organic acids | Urbanczyk-Wochniak and Fernie (2005) |
| N stress, *Eisenia veneta* (1), *Lumbricus terrestris* (2) | $^1$H NMR, PEM polar metabolites | ↑ Glutamate, citrate, isoleucine, aspartate<br>↓ Lysine, threonine | Warne et al. (2001) |
| Salt stress | | | |
| *Pseudomonas aeruginosa* | $^1$H NMR, extracellular PEM polar metabolites | ↑ Tetrahalose, glycine, betaine, valine, choline | Behrends et al. (2010) |
| *Mytilus edulis* | FT-IR, MF polar metabolites | Salinity changes metabolic fingerprinting | Bussell et al. (2008) |
| *Vitis vinifera* | GC-MS, PEM polar metabolites | | Cramer et al. (2007) |
| *Oriza sativa* | **$^1$H NMR, polar and non-polar metabolites** | ↑ Glucose, glutamate, glutamine, valine, lactose, threonine | Fumagalli et al. (2009) |
| *Limanium latifolia* | HPLC, GC-MS, $^1$H NMR, PEM polar metabolites | ↑ Proline, inorganic salts, succinate, hexoses | Gagneul et al. (2007) |
| *Lycopersicum esculentum* | FT-IR spectrometry, MF | Different metabolome fingerprinting at different salinity levels | Johnson et al. (2003) |
| *Arabidopsis thaliana* | HPLC, GC-MS, PEM polar metabolites | ↑ Lignin biosynthesis and methylation, sucrose catabolism | Kim et al. (2007) |
| *Lycopersicum esculentum* | FT-IR spectrometry, MF | Different metabolome fingerprinting at different salinity levels | Smith et al. (2003) |

**Table 1** continued

| Species | Analytical techniques, study type | Main results | Reference |
|---|---|---|---|
| *Hordeum vulgare* | GC-MS, PEM polar metabolites | ↑ Hexose phosphates, TCA cycle intermediates, GABA, proline, putrescine, several amino acids | Widodo et al. (2009) |
| Atmospheric changes | | | |
| N deposition, *Calluna vulgaris*, *Gallium saxatile* | FT-IR, MF | Changes in metabolome at different levels of N deposition | Gidman et al. (2005, 2006) |
| ↑ [$CO_2$], *Betula pendula* | HPLC, PEM polar metabolites (Phenolics) | ↑ Phenolics | Huttunen et al. (2008) |
| ↑ [$CO_2$], *Arabidopsis thaliana* (different genotypes) | GC-MS, PEM polar metabolites | Global changes in polar metabolome due to different ecotypes and also to different levels of [$CO_2$] | Li et al. (2006) |
| Organic pollutants | | | |
| ↑ (Paraquat, pyrenophorol, mesotrione, norflurazon), *Lemma minor* | $^{1}$H NMR, MF | Different changes in the metabolome fingerprinting observed as a consequence of different xenobiotics | Aliferis et al. (2009) |
| ↑ Glyophosphate, *Aporrectodea caliginosa* | $^{31}$P NMR and $^{1}$H NMR, PEM polar metabolites | ↓ Phosphalombricine, lombricine | Bon et al. (2006) |
| ↑ 3-Fluoro-4-nitrophenol, *Eisenia veneta* | $^{1}$H NMR, PEM polar metabolites | ↑ Acetate, malonate | Bundy et al. (2001) |
| ↑ (4-Fluoroaniline (1), 3,5-difluoro aniline (2), 2-fluoro-4-methylanyline 3)) *Eisenia veneta* | $^{13}$C NMR and $^{1}$H NMR, HPLC-DAD, PEM polar metabolites | ↓ Mallose (1), 2 hexhyl-5-ethyl-3-furanosulfonate (2,3) | Bundy et al. (2002) |
| ↑ 17-a-Ethynylestradiol, *Pimepheles promelas* | **$^{1}$H NMR, polar and non-polar metabolites** | ↑ Creatine, glycogen, glucose, lactate | Ekman et al. (2008) |
| ↑ Atrazine (1), fluoranthene (2), *Lumbricus rubellus* | $^{1}$H NMR, PEM polar metabolites | ↑ Fumarate (1), cytidine triphosphate (2) | Guo et al. (2009) |
| ↑ Diethanolamine (DEA) *Calanus finmarchicus* | $^{1}$H NMR, PEM polar metabolites | ↓ Choline, phosphocholine, glycerophosphocholine, taurine, sarcosine, alanine, arginine, leucine, glutamine, metionine, threosine | Hansen et al. (2010) |
| ↑ Pyrene, *Lumbricus rubellus* | **$^{1}$H NMR, GC-MS polar and non-polar metabolites** | ↑ Alanine, leucine, valine, isoleucine, lysine, tyrosine, methionine<br>↓ Tetradecanoic acid, hexadecanoic acid, octadecanoic acid | Jones et al. (2008a) |
| ↑ Chlorpyritos, *Mytillus galloprovincialis* | $^{1}$H NMR, PEM polar metabolites | ↑ Acetilcholine | Jones et al. (2008b) |
| ↑ Prometryn, *Scenedesmus vacuolatus* | **GC-MS, polar and non-polar metabolites** | ↓ Catabolic respiratory metabolism | Kluender et al. (2009) |
| ↑ (DDT, endosulfan), *Eisenia fetida* | $^{1}$H NMR, GC-MS PEM polar metabolites | ↑ Alanine | Mckelvie et al. (2009) |
| ↑ Estrogens, *Oncorhynchus mykiss* | **$^{1}$H NMR, polar and non-polar metabolites** | ↑ Vitellogenin, glyceryl<br>↓ Alanine, cholesterol | Samuelsson et al. (2006) |

**Table 1** continued

| Species | Analytical techniques, study type | Main results | Reference |
|---|---|---|---|
| ↑ (Glucosinalate, sulcotrione, AE944, furamsulfuron, benfuresate, glyphosate) *Arabidopsis thaliana* | GC-MS, PEM polar metabolites | Some metabolites changed in a different way depending on the herbicide applied; these metabolites were: glucose, fructose, metionine, phenylalanine, isoleucine, valine, lysine, tyrosine, glycine, glutamine, glyceric acid | Trenkamp et al. (2009) |
| ↑ (Atrazine, lindane), *Mytilus edulis* | $^{1}$H NMR, PEM polar metabolites | ↑ Alanine ↓ betaine, homarine, taurine (lindane)<br>↑ Leucine, isoleucine (atrazine) | Tuffnail et al. (2009) |
| ↑ Dinoseb, *Oryzias latipes* | $^{31}$P NMR and $^{1}$H NMR, HPLC-DAD, PEM polar metabolites | ↑ Ortophosphate<br>↓ ATP, phosphocreatine | Viant et al. (2006a) |
| ↑ (Dinoseb, diazinen, esfenvalerate), *Oryzias latipes* | $^{1}$H NMR, PM polar metabolites | ↓ ATP, phosphocreatine | Viant et al. (2006b) |
| ↑ 3-Trifluoro-methyl-aniline, *Eisenia veneta* | $^{1}$H NMR, PEM polar metabolites | ↑ Alanine, glycine, asparagine, glucose, citrate, succinate | Warne et al. (2000) |
| ↑ Dibenzanthracene, *Gasterosteus aculeatus* | $^{1}$H NMR, PEM polar metabolites | ↑ Malonate, glutamine, alanine<br>↓ Taurine | Williams et al. (2009) |
| Trace elements | | | |
| Cd, *Silene cucubalus* | $^{1}$H NMR, PEM polar metabolites | ↑ Malic acid and acetate<br>↓ Glutamine | Bailey et al. (2003) |
| Diverse heavy metals, *Lumbricus rubellus* | $^{1}$H NMR, PEM polar metabolites | ↓ Histidine, methylhistidine | Bundy et al. (2004) |
| Zn, *Lumbricus rubellus* | $^{1}$H NMR, PEM polar metabolites | ↑ Tryptophan, uracil, scyllo-inositol, AMP, ADP | Bundy et al. (2007) |
| Cu, *Lumbricus rubellus* | **$^{1}$H NMR, polar and non-polar metabolites** | ↓ Glucose, mannose, 3.methylhistidine | Bundy et al. (2008) |
| Cu, *Eisenia andrei*, *Lumbricus rubella* | $^{1}$H NMR, PEM polar metabolites | ↓ Histidine | Gibb et al. (1997) |
| Cd, *Clethrionomys glareolus* kidney | $^{1}$H NMR, PEM polar metabolites | ↑ Lactate and fatty acids<br>↓ Glutamine | Griffin et al. (2000) |
| $As^{3+}$, *Clethrionomys glareolus* | **$^{1}$H NMR, polar and non-polar metabolites** | Changes in lipid and glutamate metabolisms | Griffin et al. (2001) |
| Cd, *Lumbricus rubellus* | $^{1}$H NMR, PEM polar metabolites | ↑ Nicotinic acid<br>↓ Succinate | Guo et al. (2009) |
| Cd, *Myodos glareolus*, *Apodemus sylvaticus* | **$^{1}$H NMR, polar and non-polar metabolites** | ↑ Lactate<br>↓ Glucose, leucine, isoleucine | Jones et al. (2007) |
| Ni, *Mytilus galloprovincialis* | $^{1}$H NMR, PEM polar metabolites | Changes in respiratory catabolism | Jones et al. (2008b) |
| $^{131}$Cs, *Arabidopsis thaliana* | $^{131}$CsNMR, $^{31}$PNMR, $^{13}$CNMR, PEM polar metabolites | ↑ Amino acids | Le Lay et al. (2006) |
| Cu, *Rattus norvegicus* | **$^{1}$H NMR, polar and non-polar metabolites** | ↑ Citrate, lactate<br>↓ Glutamine, taurine | Lei et al. (2008) |
| B, *Hordeum vulgare* | GC-MS, PEM polar metabolites | ↑ Glycerine, putrescine, valine, fumaric acid, maleic acid, glucose, fructose | Roessner et al. (2006) |

**Table 1** continued

| Species | Analytical techniques, study type | Main results | Reference |
|---|---|---|---|
| Cd, *Arabidopsis thaliana* | HPLC-MS/MS, PEM polar metabolites | ↑ Production of proteins phytochelatins | Sarry et al. (2006) |
| Cd, *Arabidopsis thaliana* | **GC-MS, polar and non-polar metabolites** | ↑ Several sugars and amino acids, α-tocopherol, campesterol, β-sitosterol, isoflavone | Sun et al. (2010) |
| Cu, *Daphnia magna* | FT-MS, PEM polar metabolites | ↑ N-acetilspermidine | Taylor et al. (2009) |
| Radiation stress | | | |
| ↓ Visible | GC-MS, $^{13}$C NMR, PEM polar metabolites | ↓ Sugars and tricarboxylic acids | Bathellier et al. (2009) |
| ↑ UV, *Medicago trunculata* | **GC–MS, HPLC-DAD, polar and non-polar metabolites** | ↑ a-Glycerophosphate<br>↓ Leucine | Broeckling et al. (2005) |
| ↑ UV, *Arabidopsis thaliana* | GC-MS, HPLC-MS/MS, PEM polar metabolites | ↓ Phenylpropanoids<br>↑ Kaempfenrol-glycoside, quercetin glycoside | Lake et al. (2009) |
| Tropospheric ozone | | | |
| ↑ [$O_3$], *Oryza sativa* | **Electrokinetic-C, polar and non-polar metabolites** | ↑ Glutamine, GABA | Cho et al. (2008) |
| ↑ [$O_3$], *Betula pendula* | **GC-MS, HPLC-DAD, polar and non-polar metabolites** | ↑ phenolics | Kontunen-Soppela et al. (2007) |
| ↑ [$O_3$], *Betula pendula* | **GC-MS, HPLC-DAD, polar and non-polar metabolites** | ↑ phenolics | Ossipov et al. (2008) |

The studies that aimed to analyze the entire metabolite spectrum (both polar and non-polar metabolites) are highlighted in *bold* type

Polar fraction: amino acids and organic acids (citric, malate, etc.), mono-, di- and trisaccharides, inositol, sucrose, phenolics

Lipophilic (non-polar) fraction: fatty acids and their derivates, hydrocarbons, alkaloids, flavonol aglycones, triterpenoids, steroids

*PEM* Partial ecometabolomic study, *MF* metabolic fingerprinting study, *HPLC* high pressure liquid chromatography, *UHPLC* ultra-high pressure liquid chromatography, *MS* mass spectroscopy, *FT-IR* Fourier transform-infrared spectroscopy, *$^{1}$H NMR* nuclear magnetic resonance of $^{1}$H, *$^{13}$C NMR* nuclear magnetic resonance of $^{13}$C, *$^{31}$P NMR* nuclear magnetic resonance of $^{31}$P, *GC* gas chromatography, *UPLC* ultra-performance liquid chromatography

**Table 2** Ecometabolomic studies that focused on biotic interaction effects on organism metabolism

| Species | Analytical techniques and study type | Main results | Reference |
|---|---|---|---|
| Plant-fungus | | | |
| *Brassica rapa, Leptosphaeria maculans, Aspergillus niger, Fusarium oxysporum* | $^{1}$H NMR, PEM polar metabolites | ↑ Flavonoids, phenylpropanoids in infested plants | Abdel-Farid et al. (2009) |
| *Lotus japonicus, Gigaspora marginata* | IR, HPLC-MS, $^{1}$H NMR, PEM polar metabolites | ↑ Strigolatones in root plant exudates are related to hyphal branching in arbuscular mycorrhizal fungi | Akiyama et al. (2005) |
| *Brachypodium distachyon, Magnaporthe grisea* | FT-IR MF MS/MS, PEM non-polar metabolites and polar fatty acids | ↓ Phosphatidyl glycerol in infested plants | Allwood et al. (2006) |
| *Lolium perenne, Neotypodium lolii* | Linear ion trap-MS, PEM polar metabolites | Detection of fungus metabolites in infested plants (mannitol, cyclic oligopeptides) | Cao et al. (2008) |
| *Vitis vinifera, Uncinula nector, Plasmopara viticola* | $^{1}$H NMR, PEM polar metabolites | ↑ Inositol, caffeic acid in infested plants | Figueiredo et al. (2008) |
| *Triticum aestivum, Fusarium graminearum* | **GC-MS, polar and non-polar metabolites** | ↑ Metahidroxycinnamic acid, myo-inositol, glucose, malonic acid, several fatty acids and malonic acid were related to fungus infection resistance in plants | Hamzehzarghani et al. (2005) |
| *Triticum aestivum, Fusarium graminearum* | **GC-MS, polar and non-polar metabolites** | Many metabolic shifts emphasized in: ↑ fatty acids, organic acids and phenolics | Hamzehzarghani et al. (2008) |
| *Helianthus annuus, Sclerotinia sclerottorum* | $^{13}$C NMR, $^{31}$P NMR, PEM polar metabolites | ↓ Sugars and amino acids in plants and fungi during infection<br>Glycerol was exclusively produced in infected plant tissues | Jobic et al. (2007) |
| *Triticum aestivum, Mycosphaerella graminicola* | $^{1}$H NMR, PEM polar metabolites | ↑ Amino acids in infested plants | Keon et al. (2007) |
| *Vitis vinifera, Phaeomoniella spp, Fomitiporia spp.* | $^{1}$H NMR, PEM polar metabolites | ↑ Phenolics, methanol, alanine, $\gamma$-aminobutyric acid (defense mechanisms),<br>↓ Carbohydrates | Lima et al. (2010) |
| *Solanum tuberosum, Phytophthora infestans, Pythium ultimum, Botrytis cinerea* | GC-MS, PEM volatile metabolites | ↑ Increases of emission and emission of new volatiles infested Plants with changes depending on the fungus species | Lui et al. (2005) |
| *Alnus incana, Frankia* spp. | $^{15}$N NMR, $^{31}$P NMR PEM polar metabolites | ↑ Alanine, glutamine, citruline, arginine, c-aminobutyric acid | Lundberg and Lundquist (2004) |
| *Glycine max, Phytophora sojae* | **GC-MS, polar and non-polar metabolites** | ↑ Lactic acid, salicylic acid<br>↓ Sugars, aa | McGarvey and Pocs (2006) |
| *Magnifera indica, Lasiodiplodia theobromae, Colletotrichum gloeosporiodes* | GC-MS, PEM volatile metabolites | ↑ Increases of emission and emission of new volatiles in infested plants that change depending on the fungus species | Moalemiyan et al. (2007) |
| *Lupinus angustifolius, Colletotrichum lupini* | HPLC-DAD, HPLC-MS, PEM phenolics | ↑ Isoflavone aglycones in infested plants | Muth et al. (2009) |
| *Triticum aestivum, Fusarium graminearum* | **GC-MS, polar and non-polar metabolites** | ↑ Putrescine, inositol, inositol phosphate, several amino acids in infected plants | Paranidharan et al. (2008) |
| *Brachypodium sistachyon, Magnaporthe grisea* | **GC-MS, polar and non-polar metabolites** | ↑ Mannitol and glycerol production in fungus with plant photosynthate is the cause for conducting hyphal growth suggesting that fungus deploys a common metabolic re-programming strategy in host species | Parker et al. (2009) |
| *Allium cepa, Erwinia carotovora* ssp. *Carotova, Fusarium oxysporum, Botrytis alli* | GC-MS, PEM volatile metabolites | ↑ Increases of emission and emission of new volatiles in infested plants that change depending on the fungus species | Prithiviraj et al. (2004) |

**Table 2** continued

| Species | Analytical techniques and study type | Main results | Reference |
|---|---|---|---|
| *Triticum aestivum*, *Stagonospora nocorum* | $^{1}$H NMR, PEM polar metabolites | Tetrahalose is necessary for sporulation during infection | Solomon et al. (2005) |
| *Medicago truncatula, Glomus intraradices* | GC-MS, HPLC-MS, HPLC-DAD, PEM polar metabolites | ▲ Isoflavones, saponins, apocarotenoids | Strack et al. (2006) |
| *Malus domestica*, *Botrytis cinerea, Mucor piriformis*, *Penicillum expansum, Monilinia* spp. | GC-MS, PEM volatile metabolites | ▲ Increases of emission and emission of new volatiles in infested plants that change depending on the fungus species | Vikram et al. (2004) |
| Plant-microbe | | | |
| *Arabidopsis thaliana, Pseudomonas syringae (Pst)* | FT-IR, MF, polar metabolites | Evidence that infection produces metabolic changes in both plants and microorganisms | Allwood et al. (2010) |
| *Medicago sativa*, Sinorh*izobium meliloti* | $^{1}$H NMR, PEM polar metabolites | Plants react to N-fixation-deficient bacteroids by decreasing organic acid synthesis and by inducing early induction of senescence | Barsch et al. (2006) |
| *Citrus sinensis*, *Candidatus liberibacter* | **Capillary electrophoresis-DAD, polar and non-polar metabolites** | ▲ Hesperidin, naringenin, quercetin and 3 other non-identified compounds | Cevallos–Cevallos et al. (2009) |
| Catharanthus rosea, phytoplasma | **$^{1}$H NMR, HPLC-MS, polar and non-polar metabolites** | ▲ Terpene indole alkaloids | Choi et al. (2004) |
| *Arabidopsis thaliana*, *Rhodococcus fascians* | GC-MS, PEM polar metabolites | ▲ Amino acids, sugars in infested plants were induced by cytokines secreted by *R. fascians* ▼ Defensive pathways in infested plants | Depuydt et al. (2009) |
| *Medicago trunculata, Lotus japonicus, Rhizobium* spp. | HPLC-DAD, GC-MS, PEM polar | ▲ Octadecanoic acid, asparagine, glutamate, homoserine, cysteine, putrescine, mannitol, threonic acid, gluconic acid and glycerol more in nodules than in the rest of the plant | Desbrosses et al. (2005) |
| *Brassica rapa, Staphyloccocus aureus, Escherichia coli, Salonella typhimurium, Shigella flexneri* | $^{1}$H NMR, PEM polar metabolites | ▲ GABA in plants infected by gram+<br>▲ Sinapoyl-malate, caffeoyl-malate acid, histidine in plants infected by gram− | Jahangir et al. (2008) |
| *Solanum lycospericum, Pseudomanas syringae* | $^{1}$H NMR, HPLC-MS, PEM polar metabolites | ▲ Rutin and phenylpropanoids | López-Gresa et al. (2010) |
| *Arabidopsis* spp., phenyl-propanoids utilizing microbes | HPLC-MS, PEM polar metabolites of rhizosphere | Detected changes in phenylpropanoids that allow information on microbes that are able to degrade polychlorinated biphenyls | Narasimhan et al. (2003) |
| Plant-virus | | | |
| *Nicotiana tabacum*, tobacco mosaic virus | $^{1}$H NMR, PEM polar metabolites | ▲ Sesqui- and di-terpenoids, α-linolenic acid analogues, 5-caffeoylquinic acid | Choi et al. (2006) |
| *Solanum lycospericum,* Citrus exocortis virioid | $^{1}$H NMR, HPLC-MS, PEM polar metabolites | ▲ Glycosilated gentisic acid | López-Gresa et al. (2010) |
| Plant-animal (herbivory, including simulated wound stress) | | | |
| *Pseudotsuga menziesii, Megastigmus spermotherophus* | HPLC-MS-MS, PEM volatile metabolites | ▲ Acid abscisic metabolism in unpollinated plants but not in pollinated plants and some temporal variations in the rest of hormones | Chiwocha et al. (2007) |
| *Brassica oleracea*, *Pieris brassicae* | HPLC-DAD, HPLC-MS/MS, PEM polar metabolites | Evidence that caterpillars metabolize some plant secondary compounds and accumulate others as a possible mechanism of defense | Ferreres et al. (2007) |

**Table 2** continued

| Species | Analytical techniques and study type | Main results | Reference |
|---|---|---|---|
| *Nicotiana attenuate*, *Manduca sexta* | GC-MS, PEM volatile metabolites | ↑ Terpenoids, hexenylesters<br>↓ Short chain alcohols | Gaquerel et al. (2009) |
| *Brassica oleracea*, *Pieris rapae* | UPLC-MS, PEM polar metabolites | Evidence that caterpillars metabolize some plant secondary compounds and accumulate others as a possible mechanism of defense | Jansen et al. (2009) |
| *Arabidopsis thalian, and specialist and generalist herbivore insects* | HPLC-MS, PEM secondary metabolism | Changes in methylation patterns of sinapoyl malate and in the ratios between thioether versus methylsulfinyl glucosinolates | Jones et al. (2006) |
| *Barnarea vulgaris*, *Phyllotreta nemorum* | HPLC-MS, PEM polar and semipolar metabolites | ↑ Saponins | Kuzina et al. (2009) |
| *Senecio jacobaea, Senecio aquaticus, Frankiniella occidentalis* | $^{1}$H NMR, PEM polar metabolites | ↑ Pyrrolizine alkaloids, jacobine, jaconine, kaempferol glucoside | Leiss et al. (2009a) |
| *Dendranthema grandofolia*, *Frankiniella occidentalis* | $^{1}$H NMR, PEM polar metabolites | ↑ Chlorogenic acid, feruloyl quinic acid | Leiss et al. 2009b |
| *Lycopersicom* spp., *Solanum* spp., *Frankliniella occidentalis* | $^{1}$H NMR, PEM polar and semipolar metabolites | ↑ Acyl sugars | Mirnezhad et al. 2009 |
| *Arabidopsis thaliana*, *Spodoptera exigua* (generalist), *Plutella xylostella* (specialist) | **$^{1}$H NMR, PEM polar and non-polar metabolites** | Several metabolites were different among different Arabidopsis populations and glucosinolate concentration decreased the generalist but not the specialist insect growth | Arany et al. (2008) |
| *Phaseolus lunatus*, *Spodoptera exigua*, *Mythimna separata*, *Tetranychus urticae* (spider) | GC-MS, PEM volatile metabolites | Different volatile signalling pathway in the insect attack than in spider attack | Ozawa et al. (2000) |
| *Prunas persica, Myzus persicae* | $^{1}$H NMR, PEM polar and semipolar metabolites | ↑ Phenolics, cyanogenic compounds | Poëssel et al. (2006) |
| *Brassica oleracea*, *Pieris rapae* | **$^{1}$H NMR, HPLC-MS, PEM polar and non-polar metabolites** | ↑ Pinoresinol | Schroeder et al. (2006) |
| *Eucalyptus sp.* | $^{1}$H NMR, PEM apolar metabolites | ↑ Flavones | Tucker et al. (2010) |
| Marsupial and insect folivorous | | | |
| *Brassica rapa*, *Plutella xylostella, Spodoptera exigua* | $^{1}$H NMR, PEM polar metabolites | ↑ Glucose, feruloyl, sinapoyl malate, gluconapin, sucrose, threonine | Widarto et al. (2006) |
| *Solanum tuberosum,* wound stress | **GC-MS, polar and non-polar metabolites** | ↑ Organic acids, sugars, amino acids, phenylpropanoids and suberin aliphatic monomers | Yang and Bernards (2007) |
| Infochemicals | | | |
| Methyl jasmonate | $^{1}$H NMR, PEM polar metabolites | ↑ Flavonoids, fumaric acid, singirin, tryptophan, valine, threonine, valine<br>↓ Malic acid, ferulyolmalate, glutamine, several sugars | Hendrawati et al. (2006) |
| *Caenorhabditis elegans* | $^{1}$H NMR, GC-MS, HPLC-MS, PEM exudate metabolites | Exudates include 36 common metabolites including sugars, amino acids and organic acids. These metabolites attract bacteries | Kaplan et al. (2009) |
| Methyl jasmonate, *Brassica rapa* | $^{1}$H NMR, HPLC-MS, PM polar metabolites | ↑ Phenylpropanoids | Liang et al. (2006a) |

**Table 2** continued

| Species | Analytical techniques and study type | Main results | Reference |
|---|---|---|---|
| Methyl jasmonate, *Brassica rapa* | **$^{1}$H NMR, HPLC-MS, polar and non-polar metabolites** | ↑ Hydroxycinnamate, glucosinolates | Liang et al. (2006b) |
| Methyl jasmonate and it precursores, *Zea mays*, *Cotesia kariyai* | GC-MS, PEM volatile metabolites | Methyl jasmonate induces attraction of insect by ↑ hexenyl acetate iit the precursor of methyl jasmonate attracted insets by ↑ α-pinene and menthol | Ozawa et al. (2008) |
| Competition | | | |
| *Skeletonema costatum*, Thalassiosira weissflogii | UPLC-MS, PEM polar exudate metabolites | Several metabolites detected in monocultures were not found in co-culturing set up, indicating either a transformation or uptake of released metabolites by competing species | Paul et al. (2009) |
| *Stereum hirsutum*, *Coprinus disseminatus, Coprinus micaceus* | TLC, GC-MS, PEM polar exhudate metabolites | ↑ 2-Methyl-2,3-dihydroxypropionic acid, pyridoxine involved in defensive mechanisms in direct contact between mycelia of different fungus species | Peiris et al. (2008) |
| Other relationships | | | |
| *Phytophhora citricota* (fungus), *Kitasatospora* spp. (bacterium) | $^{1}$H NMR, FT-MS, PEM polar and semi-polar metabolites | Polyketide cycloheximide was found to be secreted by actinobacteria and has strong antibiosis effect against fungus | Haesler et al. (2008) |
| *Medicago x varia, Knautia arvensis, Lotus corniculatus, Bellis perennis, Leontodon autumnalis (effect of biodiversity)* | GC-MS, LC-FT-MS, PEM polar and semi-polar metabolites | Species richness produces different metabolomic shifts in the studied species. Changes are different depending on the species | Scherling et al. (2010) |
| *Salmo salar* (animal), *Aeromanas Samonicida* (bacterium) | $^{1}$H NMR, PEM polar metabolites | ↑ Acetylcholine, phosphotidylcholine, methylamine pathway ↓ Betaine, cholesterol, α- β-carbohydrates | Solanky et al. (2005) |
| Animal behavior | | | |
| Migration, *Schistocerca gregaria* | $^{1}$H NMR, PEM polar metabolites | ↑ Putrescine | Lenz et al. (2001) |
| Migration, *Schistocerca gregaria* | $^{1}$H NMR, PEM polar metabolites | ↑ L-Dopa analogue | Miller et al. (2008) |
| *Caenorhadditis elegans* | $^{1}$H NMR, HPLC-MS, PEM exudate metabolites | ↑ Ascarosides regulated sexual synchronism between male and female | Pungaliya et al. (2009) |
| *Caenorhadditis elegans* | $^{1}$H NMR, GC-MS, PEM exudate metabolites | ↑ Ascarosides increased mating and development | Srinivasan et al. (2008) |

The studies that aimed to analyze the entire metabolite spectrum (both polar and non-polar metabolites) are highlighted in *bold* type

Polar fraction: amino acids and organic acids (citric, malate, etc.), mono-, di- and trisaccharides, inositol, sucrose, phenolics

Lipophilic (non-polar) fraction: fatty acids and their derivates, hydrocarbons, alkaloids, flavonol aglycones, triterpenoids, steroids

*PEM* partial ecometabolomic study, *MF* metabolic fingerprinting study, *HPLC* high pressure liquid chromatography, *MS* mass spectroscopy, *TLC* thin layer chromatography, *FT-IR* Fourier transform-infrared spectroscopy, *$^{1}$H NMR* nuclear magnetic resonance of $^{1}$H, *$^{13}$C NMR* nuclear magnetic resonance of $^{13}$C, *$^{31}$P NMR* nuclear magnetic resonance of $^{31}$P, *GC* gas chromatography, *UPLC* ultra-performance liquid chromatography

as cryoprotectors (Carpenter and Crowe 1988). In addition other studies of partial ecometabolomics mainly focusing on lipids have reported an increase in the degree of lipid unsaturation related to cellular membrane adaptation to low temperatures (Cyril et al. 2002; Lindberg et al. 2005). When partial metabolomic studies have been conducted with transcriptomics, it has been observed that the changes in transcript levels of some metabolic processes were not correlated with shifts observed at metabolic levels (Kaplan et al. 2007). This indicates that metabolome shifts are more sensitive than transcriptome changes to detect phenotypic responses to cold stress. Different genotypes of Arabidopsis presented significant differences in the metabolite set that increased under cold acclimation (Kaplan et al. 2004; Davey et al. 2009). The metabolomic technique showed greater sensitivity in detecting genotypic and phenotypic differences to cold response than transcriptomic techniques.

Ecometabolomic studies of polar metabolites in animals in response to cold stress have reported similar results to those observed in plants, e.g., increases in glucose concentration coupled to decreases in glycogen have been observed in *Lumbricus rubellus* (Bundy et al. 2003) (Table 1). These results further confirm the increase in sugars and amino acids as cryoprotectors under low temperatures (Overgaard et al. 2007). Michaud and Denlinger (2007) conducted an ecometabolomic study using both GC-MS and $^{1}$H NMR, including both polar and non-polar metabolites. This gave a clearer picture, showing the enhancement of some metabolic pathways was related to the simultaneous decrease in other metabolic pathways. Apart from sugars, pyruvate and urea also increase at low temperatures. All these metabolites have been related to cell membrane cryoprotection (Story and Storey 1983; Lee 1991).

Contrary to cold stress, warming stress increases the saturation level of fatty acids (Larkindale and Huang 2004), and similarly to cold stress, warming stress increases amino acid and soluble sugar concentrations in plants (Kaplan et al. 2004; Rizhsky et al. 2004; Yamakawa and Hakata 2010), fungi (Pluskal et al. 2010), and the endo- and exometabolome of soil microbes (Coucheney et al. 2008) (Table 1). In animals a decrease in metabolic conditions, i.e., lower ATP and glucose concentrations (Viant et al. 2003), and a rapid increase in concentration of some amino acids with a subsequent decrease (Malmendal et al. 2006) have been linked to an increase in "heat shock" protein synthesis (Feige et al. 1996). Another recent study of Folsomia candida showed a decrease of different amino acid contents in response to heat stress as a result of upstream downregulation of transcription and translation (Waagner et al. 2010). However, the possible immediate increase of other amino acid contents was not measured. An ecometabolomic fingerprinting study of *Oncorrhynchus mykiss* eggs submitted to warming shock permitted the detection of differences in some groups of metabolites, showing the sensitivity and suitability of fingerprinting the metabolome as a screening method prior to conducting ecometabolome profile studies (Turner et al. 2007). Pluskal et al. (2010) also detected many changes in Schizosaccharomyces pombe secondary metabolism, including decreases in urea cycle intermediates and increases in acetylated compounds.

Some studies have monitored the changes in the concentration of water soluble plant metabolites in response to water availability to study drought effects using partial ecometabolomic techniques in vitro or in the lab (Rizhsky et al. 2004; Pinheiro et al. 2004), greenhouse (Cramer et al. 2007; Charlton et al. 2008; Lugan et al. 2009) and also field experimental conditions (Semel et al. 2007; Mane et al. 2008; Alvarez et al. 2008) (Table 1). An increase in soluble sugars, mainly glucose and sucrose and/or in amino acids or their derivates (citric acid, threonine, homoserine, valine, proline, malate, $\gamma$-aminobutyrate) is frequently found under drought conditions, confirming that the reduction in photosynthesis is accompanied by a pronounced mobilization of sugars in soluble form and by a synthesis of soluble amino acids. Both these mechanisms adjust osmotic potential to prevent water losses. These compounds may also protect cellular components such as membranes and enzymes (Shen et al. 1997). A similar observation has been reported by Michaud et al. (2008) in a partial ecometabolomic study of the polar metabolites of the insect *Belgica antartida* raised in vitro at different moisture levels (Table 1). Unfortunately, significant plant secondary metabolites, such as phenolics, that can minimize the oxidative stress associated with drought (Hura et al. 2007) were not included in such studies. For this reason ecometabolomic studies that aim to study primary but also secondary metabolites at once are necessary to reach a general understanding of plant responses to drought.

The effects of seasonality on the leaf content of some metabolites have been observed in some target studies (Riipi et al. 2004) in mountain birch trees (*Betula pubescens*). Ecometabolomic studies should allow us to improve our knowledge of global metabolism shifts linked to annual phenological changes in both animals and plants in field conditions.

## Nutrient deficiency

Ecometabolomic studies that have aimed to investigate metabolome changes under different scenarios of nutrient availability are scarce (Table 1). N deficiencies in plants have been proven to decrease certain amino acid concentrations, whereas the concentration of several sugars,

phosphoesters and secondary metabolites increases (Urbanczyk-Wochniak and Fernie 2005; Bölling and Fiehn 2005) (Table 1). There are also shifts from biosynthesizing some amino acids to synthesizing others (Howarth et al. 2008). These effects were also observed in the earthworm *Eisenia veneta* (Warne et al. 2001). Partial ecometabolomic studies of polar metabolites found that **P stress** induced the root accumulation of polyols, which are stress-related metabolites (Hernández et al. 2007). In addition plants modify carbohydrate metabolism in order to reduce P consumption and remove P from many metabolites (glucose 6-P, fructose 6-P, inositol 1-P and glycerol 3-P), thus reducing the levels of organic acids involved in the tricarboxylic acid cycle (TCA) (Huang et al. 2008) (Table 1). Partial ecometabolomic studies in plants have shown that S deficiency tends to increase the concentration of some N-rich metabolites such as certain amino acids and purines (Nikiforova et al. 2005a; Howarth et al. 2008). Rochfort et al. (2009) have observed that changes in both polar and lipophilic metabolite composition in earthworms provides information on the fertility of the soil in which they have been living.

### Salt stress

Plant salt stress is increasing globally because of climate change and inappropriate water use by humans and field management (Ellis and Mellor 1995). Current reports using metabolomic techniques have detected the global metabolic shift under salt stress observing the sets of metabolites that more frequently increased, enhancing the plant osmotic potential. Inorganic solutes (Gagneul et al. 2007) and sugars, amino acids and polyols (Kim et al. 2007; Fumagalli et al. 2009; Widodo et al. 2009; Behrends et al. 2010) are the metabolites that most frequently increase under salt stress (Table 1). Some studies have investigated the metabolomic fingerprinting studies of salt-stressed plants (Smith et al. 2003; Johnson et al. 2003) and animals (Bussell et al. 2008), and have been able to identify the molecular groups changing under such stress (Table 1).

### Hypoxia

Lack of oxygen is an important ecological trait in some ecosystems such as benthonic communities in intertidal coastal areas. Increases in succinate and valine and decreases in leucine and isoleucine have been observed in $^{1}$H NMR partial ecometabolomic studies of polar metabolites in the mussels *Mytilus edulis* and *Mytlus galloprovincialis* grown at different hypoxia levels (Hines et al. 2007; Tuffnail et al. 2009) (Table 1). The fish *Oryzias latipes* has shown increases of soluble phosphates and decreases in ATP and phosphodiesters in response to hypoxia in a PEM of phosphorylated soluble metabolites by $^{31}$P NMR (Pincetich et al. 2005).

### Global change drivers

Changes in atmospheric composition ($CO_2$, $O_3$, $NO_x$), warming, drought, human-made pollution, trace element pollution and UV radiation can have a strong impact on organism metabolomes. The effects of these abiotic factors on organism metabolites have been currently investigated in some ecometabolomic studies. Metabolites linked to carbohydrate biosynthesis and partitioning, amino acid metabolism, cell wall and hormone biosynthesis pathways were the most affected by [$CO_2$] increases in different genotypes of *Arabidopsis thaliana* submitted to higher [$CO_2$] (Li et al. 2006). Thus, the responses of the most polar metabolite groups were different depending on the genotypes. However, the use of transcriptome allowed detecting that there were a small number of signature transcripts that appeared as a common response mechanism of all Arabidopsis ecotypes to [$CO_2$] increases irrespective of their underlying genetic diversity and evolutionary adaptation to different habitats (Li et al. 2006). Thus, these results highlight the advantage of using metabolomic with other omic techniques at once to reach a general and integrated overview of the responses of organisms in response to environmental changes.

Increases of phenolic compound contents have been observed in target analysis at high [$CO_2$] exposure (Huttunen et al. 2008) (Table 1). This linkage between high [$CO_2$] environmental concentrations and high C-rich compound concentrations warrants being studied together with the whole organism metabolome. Such rises in C-rich secondary metabolites in plant tissues seem related to the increases in the plant C:N concentration ratio observed as a general plant response to higher [$CO_2$] (Peñuelas et al. 1997; Novotny et al. 2007).

Ecometabolomic fingerprinting using Fourier transformed-infrared spectroscopy FT-IR has been used to investigate patterns of plant metabolome changes due to N deposition. This was done both in an experimentally manipulated N deposition gradient under common garden conditions using *Calluna vulgaris* and in a natural gradient across the United Kingdom (Gidman et al. 2005, 2006) (Table 2). In both studies FT-IR fingerprinting was able to correlate metabolome changes with N deposition levels. The results showed that the spectra regions corresponding to N–H, C–N, proteins and polysacarid vibrational bands had a positive relationship with higher N-deposition, suggesting an enhancement of N-rich metabolite synthesis and of sugar anabolism. These results highlight the possiblities of the use of this technique as a useful tool for preliminary

studies in metabolome research applied to field ecological studies.

Partial ecometabolomic studies allow the detection of the metabolic pathways affected by the pollution produced by different chemotoxic pollutants and also the key metabolites that improve the organism resistance both in plants (Trenkamp et al. 2009; Kluender et al. 2009) and in animals (Warne et al. 2000; Bundy et al. 2001; Viant et al. 2006a, b; Samuelsson et al. 2006; Bon et al. 2006; Jones et al. 2008a; Ekman et al. 2008; Mckelvie et al. 2009; Tuffnail et al. 2009; Hansen et al. 2010) (Table 1). For instance, Bundy et al. (2002) have used ecometabolic fingerprinting NMR studies of the earthworm *Eisenia veneta* to ascertain initially the metabolites that changed their concentration when the worm was submitted to three different xenobiotics: 4-fluoroaniline, 3,5-difluoroaniline and 2-fluoro-4-methylaniline. In a later step they identified these changing compounds by using HPLC–MS and $^{13}C$ NMR. This experiment is an example of the utility of metabolic fingerprinting studies to detect the molecular groups which change in the metabolome in response to an abiotic factor as a first step prior to molecular qualitative determination analyses. Using this approach avoids the expense and time consumption that qualitative analyses of the whole metabolome require. Hansen et al. (2010) showed the shifts in metabolism of the marine copepod *Calanus finmarchicus* in response to diethanolamine (DEA) exposure as a relevant chemical widely used in industrial, agricultural and pharmaceutical applications. The main result of this study was a decrease of choline, taurine, sarcosine and some amino acids. Likewise, the capacity of ecometabolomic fingerprinting to detect metabolomic responses to different herbicide exposures has been recently reported in *Lemna minor* (Aliferis et al. 2009). In the metabolomic studies of toxicity effects on an organism's metabolome (polar and non-polar metabolites), those using GC-MS have been able to detect more compounds than those using $^{1}$HNMR. In contrast, studies using $^{1}$HNMR have greater power to elucidate the compound structure through its great number of sources of qualitative determination (COSY, TOCSY and high-resolution magic angle spinning). Thus, if the aims are also to determine possible novel structures, the use of $^{1}$HNMR is advisable. For example, Kluender et al. (2009) using GC-MS detected 283 metabolites but determined only 39, whereas Jones et al. (2008a) using $^{1}$HNMR detected and determined 32 analytes in a similar study on chemotoxic pollutant effects on the invertebrate metabolome. Jones et al. (2008a) using both techniques to analyze the same samples detected and determined 32 molecules using $^{1}$HNMR and detected 51, but only determined 42 molecules using GC-MS.

A set of recent partial ecometabolomic studies of polar metabolites has also permitted the identification of the metabolites and metabolic pathways affected by certain trace element polluting plants (Bailey et al. 2003; Roessner et al. 2006; Sarry et al. 2006; Le Lay et al. 2006; Sun et al. 2008) and animals (Gibbs et al. 1997; Griffin et al. 2001, 2000; Bundy et al. 2007, 2004; Jones et al. 2008b; Taylor et al. 2009; Guo et al. 2009) (Table 2). These studies have allowed the identification of some metabolites (acetilspermidine, glucose, histidine, l-methylhistidine, mannose) that can be used as biomarkers for Cu, Cd and Zn pollution in various animal species (Bundy et al. 2004, 2007, 2008; Taylor et al. 2009; Guo et al. 2009). In rats, in addition to the changes in amino acid and sugar contents, a further abnormal metabolome composition has been observed as a result of Cd and Cu intake (Jones et al. 2007; Lei et al. 2008), and changes in lipid metabolism under high levels of As have also been observed (Griffin et al. 2000). A tendency to increase the production of AMP, ADP and N-α-methylhistidine, and to generate an imbalance in metabolites related to energetics by reducing ATP levels has been reported in *Lumbricus rubellus* (Bundy et al. 2007, 2008). In a transcriptomic and ecometabolomic study of Cu toxicity in *Lumbricus rubellus*, Bundy et al. (2008) observed a decrease in small-molecule metabolites (glucose and mannose) related to an overexpression of transcripts of enzymes involved in oxidative phosphorylation, thus showing that Cu interferes with energy metabolism. In addition such studies have advanced the knowledge about the metabolites involved in phytochelatin mechanisms in plants (Sarry et al. 2006). Pollution by $^{133}Cs$ induced higher amino acid content in cells of *Arabidopsis thaliana*, suggesting an induction of the synthesis of abnormal or unwanted proteins under higher levels of $^{133}Cs$ in the medium. On the other hand, the synthesis increase of enzymes involved in the degradation of short-lived intracellular proteins was detected by proteomic studies (Le Lay et al. 2006). These effects were lower at high levels of K in the growth medium. Altogether these results highlighted the benefit of coupling different omics approaches together with the simultaneous study of different environmental variables such as pollution and nutrient availability levels in the Le Lay et al. (2006) study. Similarly, an increase of some amino acid contents has been observed in *Silene cucubalus* and *Arabidopsis thaliana* submitted to Cd pollution (Bailey et al. 2003; Sun et al. 2010) and in *Hordeum vulgare* submitted to B pollution (Roessner et al. 2006), all supporting the Le Lay et al. (2006) study results.

High levels of UV radiation can be expected in the future if stratospheric $O_3$ levels decrease. This type of radiation is able to damage DNA and other macromolecules (Harm 1980; Jagger 1985). Several target studies have already observed that *Quercus ilex* and *Rhododendrum ferrugineum* change their pigment composition and

leaf morphology in response to UV radiation in a latitudinal range (Filella and Penuelas 1999) demonstrating that a deep metabolic change can also occur, but these studies do not allow us to reach a global picture of metabolome shift under UV radiation enhancement. Partial ecometabolomic studies carried out exposing Arabidopsis thaliana to high UV radiation showed that flavonoids (Lois 1994), α-glycerophosphates (Broeckling et al. 2005) and phenylpropanoids (Lake et al. 2009) increased their concentrations, thus suggesting that these compounds have a photoprotective role (Table 2). In a partial ecometabolomic study of polar metabolites under light starvation using GC-MS and $^{13}C$ NMR, a decrease in primary metabolism has been observed in roots of *Phaseolus vulgaris* (Bathellier et al. 2009). But in this field there is a lack of global ecometabolomic studies aiming to study polar and non-polar metabolites at one time to improve our knowledge of the plant and animal capacity and mechanisms to respond to increasing levels of UV radiation.

Some interesting ecometabolomic studies including polar and non-polar metabolites have already begun to contribute to providing information about the effects of ozone ($O_3$) on the organism metabolome of different taxa. In an ecometabolomic study of rice (*Oryza sativa*) using electrokinetic chromatography, increases in $O_3$ concentrations were found to enhance γ-aminobutiric acid (GABA), some amino acids and glutathione (Cho et al. 2008) (Table 1). However, when ecometabolomic studies were conducted using GC-MS in the tree *Betula pendula* as the target species growing in common garden conditions, phenolics and compounds related to the leaf cuticular wax layer were found to be the main metabolites involved in metabolic adaptation to increasing $O_3$ levels (Kontunen-Soppela et al. 2007; Ossipov et al. 2008) (Table 1). These are the first contributions to understanding what metabolic compounds and metabolic pathways are involved in phenotypic responses to $O_3$ pollution, and they highlight that plants can respond to $O_3$ pollution at different metabolic levels, which can vary between species.

### Remaining questions

This overview of the studies that have used ecometabolomic techniques to study ecophysiological responses to changes in abiotic variables shows there are several key questions still to be tackled. (1) The abiotic variables studied have been mainly manipulated in controlled conditions. Few studies have been conducted observationally in natural gradients or experimentally in field setups. (2) There is a lack of ecometabolomic studies with polar and non-polar metabolites and also considering the secondary metabolism in plants. (3) There is a lack of information about the response of several taxa or ecotypes, such as vertebrates, trees or shrubs, and there is an insufficient number of comparable studies enabling more general conclusions. (4) Although there are preliminary studies, such as detecting changes in the metabolome in different soils and climatic conditions (Wallenstein et al. 2010), ecometabolomic studies of multifactorial effects are just beginning.

## Biotic interactions between two or more species

### Plant-fungus

Few studies have applied ecometabolomic techniques to study changes in the plant metabolome during fungal infection, which can be a host-pathogen or a mutualistic relationship. Some partial ecometabolomic studies of polar metabolites have shown that plant defensive responses involve the induction of diverse polar secondary metabolites such as p- and m-coumaric acid, inositol, caffeic acid, indolic derivates, phenylpropanoids and flavonoids, and some non-polar metabolites such as phosphatidyl glycerol, aromatic compounds and fatty acids (Bednarek et al. 2005; Allwood et al. 2006; Strack et al. 2006; Jobic et al. 2007; Cao et al. 2008; Hamzehzarghani et al. 2008; Muth et al. 2009; Abdel-Farid et al. 2009; Lima et al. 2010) and also the decrease of other metabolites such as GABA, fructose and sucrose involved in plant growth and primary metabolism (Jobic et al. 2007; Abdel-Farid et al. 2009) (Table 2). Similarly, higher contents of some amino and organic acids, carbohydrates and mainly of phenolic compounds have been observed to be constitutive defenses in fungus-resistent subspecies of *Vitis vinifera* when compared with susceptible subspecies of *Vitis vinifera* (Figueiredo et al. 2008; Ali et al. 2009). These results imply a rerouting of carbon and energy from primary to secondary metabolism and suggest the possible change in the plant nutritional quality for animals feeding with a possible impact on other ecological relationships. The other component of the relationship, the fungus, also changes its metabolism when it infests a plant. For example, Parker et al. (2009) suggested that the fungus *Magnaporthe grisacea* deploys common metabolic pathways of the host plant species *Brachipodium sistachyon* to suppress plant defenses and colonize plant tissues. In this vein Jobic et al. (2007) found that glycerol was only produced for the hyphae of the fungus *Sclerotinia sclerotiorum* when it grew into infected tissues of *Helianthus annuus*. Glycerol can be synthesized by fungus to enhance fungal growth and to protect fungal cells. Metabolites released from dying plant tissues could stimulate its synthesis. These ecometabolomic studies show not only the metabolites induced in plants by fungal infection (Hamzehzarghani et al. 2005, 2008; Muth et al. 2009;

Abdel-Farid et al. 2009; Lima et al. 2010), but also shows that the fungus can use plant photosynthate that thereafter causes hyphal growth, altogether suggesting that the fungus has developed a common metabolic re-program strategy in the plant host (Parker et al. 2009). Another interesting finding reported by four different studies is that fungal infection induces the emission of new volatile organic compounds (VOC) with a composition that varies depending on the species of infecting fungus (Prithiviraj et al. 2004; Vikram et al. 2004; Lui et al. 2005; Moalemiyan et al. 2007) (Table 2). VOC has been shown to have direct and indirect roles in protecting plants against herbivory (Llusia and Peñuelas 2001; Peñuelas and Llusia 2004), as well as in contributing to the plant's defense strategies against thermal damage (Copolovici et al. 2005; Peñuelas et al. 2005a). Thus, these ecometabolomic studies of plant emissions due to fungus infection suggest a role of defense coordination among plant organs and/or among different individual plants at the community level.

Fungus can also establish symbiotic relationships with plants to enhance the nutrient and water absorption capacity. Akiyama et al. (2005), using HPLC-MS and $^{1}$H NMR ecometabolomic studies of root exudates, found that Lotus japonicus exudes sesquiterpene lactones, stimulating the hyphal branching of the symbiotical fungus *Giaspora margarita*. Cao et al. (2008) studied the symbiotic relationship between *Lolium perenne* and the endophytic fungus *Neotyphodium lolii* using mass spectrometry. The ecometabolomic study showed that plants with fungus symbionts contain some fungal compounds such as peramine, mannitol and other metabolites in their metabolome as a result of their relationship with the fungus. Some of these metabolites have been identified as plant-endophyte symbiotic metabolism regulation, but its role is not yet stablished. All hypotheses suggest a possible role in the improvement of the resistance to biotic or abiotic factors such as herbivores or drought (Cao et al. 2008). Thus, the current ecometabolomic studies in plant-fungus symbiotic relationships are promising for a better comprehension of the metabolic mechanisms underlying the symbiosis success in field conditions.

### Plant-bacteria and plant-virus

Similar to plant-fungus, the relationship between plants and microbes can be either a host-pathogen relationship or a mutualistic relationship. Shifts in metabolome fingerprinting have been described in host plant-pathogen bacterial relationships (Allwood et al. 2010). Some partial ecometabolomic studies of polar metabolites have shown that bacterial infection induces chemical defensive mechanisms involving an increased synthesis of diverse metabolites such as threonine, sinapoyl-malate, caffeoyl-malate, $\gamma$-aminobutic acid, rutin or phenylpropanoids depending on the species (Barsch et al. 2006; Jahangir et al. 2008; López-Gresa et al. 2010) (Table 2).

Pathogen bacteria can change plant metabolism. In this way, studies of polar and non-polar metabolites have reported that some primary plant metabolites increased their concentration under microbe infection, but also that some secondary metabolite pathways were also stimulated by microbe infection, e.g., terpenoid indole alkaloids and phenylpropanoids (Choi et al. 2004) and flavonoids (Cevallos-Cevallos et al. 2009) (Table 2). Ecophysiological studies using metabolomic techniques have also shown that they are able to detect differences in plant metabolism shift depending on the virulence (Simoh et al. 2009) or the nutritional requirements (André et al. 2005) of the microbe strain. Ecometabolomic studies can also help to discern what environmental conditions are more or less favorable for the success of the symbiotic relationships. Barsch et al. (2006) have observed that the host plant *Medicago sativa* reacts against nitrogen-fixation-deficient bacteroids with a decrease of organic acid synthesis and an early induction of their senescence.

The symbiotic relationships between some plant taxons, such as the Fabaceae, with certain anaerobic bacteria are of great ecological and farming importance. The few metabolic studies available on this topic have observed that plants induced nodule formation by increasing the synthesis and nodule accumulation of particular metabolites such as asparagine, glutamate, putrescine, mannitol, threonic acid or gluconic acid (Desbrosses et al. 2005).

Studies to improve the ecological tools for environmental restoration are also an emerging area. In this field, Narasimhan et al. (2003) in a rhizosphere study using an HPLC-MS partial ecometabolomic analysis of rhizosphere secondary metabolites reported that phenylpropanoid-utilizing microbes are able to enhance soil polychlorinated biphenyl (PCB) depletion. This is useful in soil phytoremediation for a successful selection of rhizosphere microbes that degrade PCBs, which are very toxic organic pollutants.

The repercussions in the metabolome of viral infections in plants in the field have been little studied. Ecometabolomics of polar metabolites suggest that plants respond similarly to bacterial infection, increasing the synthesis of organic acids and terpenoids (Choi et al. 2006; López-Gresa et al. 2010).

### Plant-animal

Plant insect interactions are one of the most widely studied topics in ecology. Ecometabolomics offers the possibility of a more in-depth study of the biochemical aspects of this interaction and of its ecological

implications. Some partial ecometabolomic studies of polar metabolites have found a great chemical variation in the metabolites used by plants to defend themselves against insect attacks (Widarto et al. 2006; Jones et al. 2006; Poëssel et al. 2006; Jansen et al. 2009; Leiss et al. 2009a, b; Kuzina et al. 2009; Mirnezhad et al. 2010) (Table 2). These studies have demonstrated that some hormones, acyl sugars and mainly different secondary metabolites such as chlorogenic acid, saponins, phenolics, terpenes, alkaloids, terpenes and glucosinolates are the most general plant chemical defenses against insect herbivores. These results further confirm that a great variety of chemicals are used by plants as chemical deterrents and also the different capacities for chemical resistance among different plant species and also among different subspecies of the same species (Mirnezhad et al. 2010). These results also suggest that the consequences at the community level of plant-insect interaction can vary depending on the taxonomy of the plant and insect as a result of the variability of the changes in palatability, stoichiometry and chemical signals. Insect attack not only affects soluble metabolites, but also the molecular composition of volatile emissions from plants (Peñuelas et al. 1995; Ozawa et al. 2000; Gaquerel et al. 2009) (Table 2). Terpenes are induced and emitted in response to internal (genetic and biochemical) and external (ecological) factors, both biotic and abiotic, and play an important role in communication among plants, and in animals have been widely observed (Peñuelas and Llusia 2001; Peñuelas et al. 2005b). Metabolome studies in specialist insects *Pieris brassicae* feeding on the leaves of *Brassica oleracea* have shown that insect growth was not affected and that the insect metabolized some flavonoids of *B. oleracea* (Ferreres et al. 2007) and accumulated certain other flavonoids (Jansen et al. 2009), suggesting a possible defense mechanism against predators. Ecometabolomic studies have also allowed observing changes in hormone concentrations of Pseudotsuga menziesii in response to attack by the insect, Megastigmus spermotrophus (Chiwocha et al. 2007). The conclusion of all these results is that ecometabolomics is a useful tool to discover unexpected bioactive compounds involved in ecological interactions between plants and their herbivores.

Arany et al. (2008) in an ecometabolomic study of polar and non-polar metabolites found that *Arabidopsis thaliana* shows that aliphatic glucosinolates and total glucosinolates affect the generalist herbivore *Spodoptera exigua*, but not the specialist herbivore *Plutella xylostella.* This study demonstrates different strategies of plant defense against generalist and specialist herbivores, although future studies are necessary to advance in this topic. In the research line of plant-herbivore relationships, Yang and Bernards (2007) have studied the metabolome shift after leaf cutting to investigate the metabolic pathways involved in suberification. Some recent studies suggest that ecometabolomic studies can also be useful to study the molecular interaction between mammals and plants (Tucker et al. 2010).

Plants synthesize complex sets of substances that interact with other plants and with animals. Ecometabolomic studies can play a key role in advancing the knowledge of the mechanisms of action of these substances on organisms. An example of this possible role comes from the information provided by several partial ecometabolomic studies that have observed increases in the contents of metabolites in terrestrial plants linked to chemical defensive and/or antistress mechanisms in response to methyl jasmonate (Liang et al. 2006a, b; Hendrawati et al. 2006). Methyl jasmonate has also proved to enhance the monoterpene emission by 20–30% in *Quercus ilex* leaves (Filella et al. 2006), suggesting that plants change their metabolism not only to enhance a direct defensive response, but also to enhance signaling to attract natural enemies of herbivores. However, we must highlight that in the most studies the animal was an insect, and there is a lack of studies with vertebrate herbivores.

## Competition

Peiris et al. (2008), using GC-MS partial ecometabolomics of polar metabolites, have studied the mycelial tissues of three competing fungus species during wood decomposition. They observed that the synthesis of 2-methyl-2,3-dihydroxypropinoic and pyridoxine acids can be involved in defensive mechanisms activated in response to direct contact between the mycelia of the different fungus species studied, thus showing the importance of exudated metabolites as resources for chemical defense in direct competition for space and sources. Recently, Paul et al. (2009) studied the interspecific competition and allelophatic effects between two diatom species, observing different metabolites in monocultures than those observed in a co-culturing setup. This indicates either transformation or uptake of released metabolites by the competing species. The effects of species richness on plant metabolomes were also studied in five herbs: two tall-growing herbs (*Medicago* x *varia* and *Knautia arvensis*) and three small-growing herbs (*Lotus corniculatus*, *Bellis perennis* and *Leontodon autumnalis*) competing in communities of different species composition and richness (Scherling et al. 2010) (Table 2). They found different metabolome shifts in plant species growing in such different communities. All these results suggest new lines of research in the frame of competition in natural ecosystems by studying the ecometabolomic relationships between competitors.

### Other biotic relationships

A functional assessment of antagonistic microbial communities in soil requires in-depth knowledge of the mechanisms involved in these interactions. Metabolome studies provide an adequate tool for this purpose. Haesler et al. (2008) found the metabolite polyketide cycloheximide to be the molecule responsible for the antagonistic effect of the actinobacterial genus *Kitasatospora* against the oomycetous root pathogen *Phytophthora citricola*.

Other important biotic relationships such as animal-microbe have been rarely studied by partial ecometabolomic methods (Solanky et al. 2005) (Table 2).

### Animal behavior

Animal processes such as migrations, mutualistic associations or reproductive strategies are species-specific traits associated with physiological and metabolic changes. In spite of this, some of these phenomena have not yet been studied using ecometabolomic approaches. *Schistocerca gregaria,* a desert locust that forms great migratory swarms, has been studied using a $^{1}$H NMR partial ecometabolomic analysis of polar metabolites. This study has shown that some metabolites like putrescine are linked to solitary behavior (Lenz et al. 2001), while others such as the alkaloid L-dopa analogue are linked to gregarious behavior (Miller et al. 2008). These studies should provide significant advances in knowledge about and predictions of the future behavior of such populations. This is of great importance for plague management. Another promising study has been conducted in the exudates of the nematode, *Caenorhadditis elegans*, with $^{1}$H NMR and has proved that ascarosides are the substances responsible for mating and sexual reproductive synchronization between males and females of this species (Srinivasan et al. 2008; Pungaliya et al. 2009). However, there is a lack of studies on other taxonomic groups than insects to reach a more global knowledge on this topic.

### Interactions between abiotic and biotic factors

Few studies have investigated the effects of abiotic factors on biotic relationships. Among them we highlight ecometabolomic studies of polar metabolites that have investigated the effects of N availability on plant-fungus infection relationships (Rasmussen et al. 2008), showing that N availability affects both plant metabolite concentrations and fungus infection (Table 3). Recently, Larrainzar et al. (2009), studying $N_2$-fixation under drought conditions, observed a decrease in amino acids and sugar concentration and in the $N_2$-fixation activity in roots of *Medicago trunculata*. Prior to this, Rosenblum et al. (2005) used a $^{1}$H NMR partial ecometabolic analysis to study the polar metabolites in red abalone, Haliotis rufenses, during infection by the rickettsia *Candidants xenohaliotis californiensis* under different levels of food sources. They concluded that food levels and other abiotic factors such as water temperature are responsible for disease development. Likewise the *Haliotis* spp. metabolome shift response induced by *Rickettsia* spp. included decreases in amino acids and carbohydrates and increases in taurine, glycine, betaine and homarine similarly at different temperatures (Rosenblum et al. 2006) (Table 3).

## Metabolomics coupled with other omics approaches

Metabolomics allows the determination of the phenotype response directly (Fiehn et al. 2000), whereas transcriptomics and proteomics analyze the way genome expression translates to biological response, thus allowing us to detect the genes that are expressed in one determined moment (Colebatch et al. 2004; Fridman and Pichersky 2005; Saito and Matsuda 2010). The simultaneous use of metabolomics with genomics, transcriptomics and/or proteomics provides a global overview from transcription to final metabolic products that allows reaching better knowledge of the regulatory networks of metabolic pathways than the omics studies used without metabolomics analysis. For instance there are many metabolomics pathways regulated at the post-transcriptional level (Kaplan et al. 2007). The integrated omic studies are specially promising for ecological studies since they make possible discerning between genotype dependent and independent response to environmental changes for multiple characters at once (Pena et al. 2010; Wienkoop et al. 2008). This provides an overview of the short-, medium- and long-term responses to environemental changes and their integrative relationships. Metabolomic databases can be combined with data sets from other "omic" technologies (Weckwerth 2008) to enhance data value and permit a system-wide analysis from genome to phenome (just as the genome and proteome signify all of an organism's genes and proteins, the phenome represents the total sum of its phenotypic traits).

Some past studies have begun to demonstrate these arguments. Metabolomics and transcriptomics can be combined and analyzed mathematically together. In this way, the metabolites and genes regulated by the same mechanisms cluster together (Table 4). For example, integrated experiments in transcriptomic and ecometabolomic studies have been successfully conducted in *Arabidopsis* at different levels of sulfur supply to elucidate gene-to-gene and metabolite-to-gene networks (Hirai et al. 2004, 2005; Nikiforova et al. 2004, 2005b; Fukushima et al. 2009b). These studies allow the detection of general and specific responses to different nutrient deficiencies and the identification of gene function with the added value of the improvement in the

**Table 3** Ecometabolomic studies involving two or more ecological relationship effects on organism metabolism

| Factors and species | Analytical techniques, study type | Main results | Reference |
|---|---|---|---|
| Volatile emissions of plant (*Zea mays*) attacked by herbivore (caterpillars to attract parasite wasps, natural enemies of the herbivores) | GC-MS, TP volatile compounds | 23 volatile compounds were identified as responses to caterpillar regurgitant injection in different *Zea mays* genotypes and the species specific emissions varied among *Z. mays* phenotypes | Degen et al. (2004) |
| Drought effects on plant (*Medicago trunculata*)-$N_2$ fixers | GC-MS, PEM polar metabolites | Drought reduced $N_2$ fixation rates and amino acid and carbohydrate concentration | Larrainzar et al. (2009) |
| N availability effect on plant (*Lolium perenne*)-fungus (*Neotyphodium lolii*) relationships | GC-MS, PEM polar and non-polar primary metabolites, flavonoids and anthocyanins | The effects of fungus on plant metabolome are great and depend on N supply levels | Rasmussen et al. (2008) |
| Effects of water, temperature and food availability on animal (*Haliotis refrescens*)-microbe (*Candidatus xenohaliotis californiensis*) relationships | $^{1}$H NMR, PEM polar metabolites | Glucose:homarine concentration ratio in foot muscle results for the metabolic marker for differentiating *Haliotis* individuals only infected compared to those both infected and also food limited | Rosenblum et al. (2005) |
| Effects of temperature on animal (*Haliotis* spp.)-microbe (*Rickettsia* spp.) relationships | $^{1}$H NMR, PEM polar metabolites | Infection increased taurine, glycine, betaine and homarine at all temperatures studied | Rosenblum et al. (2006) |

Polar fraction: amino acids and organic acids (citric, malate, etc.), mono-, di- and trisaccharides, inositol, sucrose, phenolics

Lipophilic (non-polar) fraction: fatty acids and their derivates, hydrocarbons, alkaloids, flavonol aglycones, triterpenoids, steroids

*PEM* partial ecometabolomic study, *MF* metabolic fingerprinting study, *HPLC* high pressure liquid chromatography, *MS* mass spectroscopy, *TLC* thin layer chromatography, *FT-IR* Fourier transform-infrared spectroscopy, *$^{1}$H NMR* nuclear magnetic resonance of $^{1}$H, *$^{13}$C NMR* nuclear magnetic resonance of $^{13}$C, *GC* gas chromatography, *UPLC* ultra-performance liquid chromatography

production of useful compounds in plants. Furthermore, some of these studies have provided novel knowledge among metabolic pathways linked to the physiological endpoint involved in plant homeostasis and responses to nutrient stress (Nikiforova et al. 2004, 2005b). Similarly coupling metabolomic with other omic approaches has allowed the improvement of our knowledge of the metabolic process and their regulation in response to other environmental factors such as salinity (Brosché et al. 2005; Gong et al. 2005), drought (Vasquez-Robinet et al. 2008), oxidative stress (Baxter et al. 2007), symbiotic $N_2$ fixation (Hernández et al. 2009; Sanchez et al. 2010), and plant-herbivore (Kant et al. 2004) and plant-microbe (Ward et al. 2010) interactions (Table 4). Similar holistic results can be expected by combining ecometabolomics with proteomics (Weckwerth 2008). For more detailed and specific information, see Macel et al. (2010), who provide detailed information about the possibilities of integration of metabolomics with other omics approaches.

## Further applications in ecology

Ecometabolomic studies may provide new perspectives and insights into many ecological topics. In an attempt to highlight the important role that metabolome studies could have in ecology, here we propose the application of metabolome studies to some critical questions currently under debate in ecology (Fig. 1).

### Stoichiometry, growth rate and other ecological hypotheses

Organisms are the products of chemical reactions, and their growth depends on the availability of various elements, especially carbon, nitrogen and phosphorus. In this context, ecometabolomic studies also provide an opportunity to make direct advances related to the ecological hypotheses that aim to study ecosystem structure and function from a chemical perspective. One those hypotheses, the growth-rate hypothesis (GRH) (Sterner and Elser 2002), links the relative element content of organisms to their growth rate, the idea being that fast-growing organisms need relatively more P-rich RNA, which is the main component of the protein-producing ribosome, in order to support rapid protein synthesis. Consequently, ecosystem conditions that produce organic matter with low C:P and N:P ratios would be expected to result in higher adaptive growth rates, more efficient energy transfer through a food web and increased biomass of large-bodied animals relative to that of small-bodied organisms (Sterner and Elser 2002). Further assessment of the GRH evidently requires many more

**Table 4** Ecometabolomic studies coupled to other omics studies

| Factors and species | Analytical techniques, study type | Main results | Reference |
|---|---|---|---|
| *Arabidopsis* sp under S stress | Metabolomics (GC-MS, PEM polar metabolites) and transcriptomics | ↓ Carbon and aa pathways and overall vision of antioxidative metabolism strategy | Baxter et al. (2007) |
| Different *Populus* sp. under drought | Metabolomics (GC-MS, PEM polar metabolites), genomic and transcriptomic | Drought increased aa contents. The most drought-adapted species did not present different genes per se than the other species, but the regulation of gene expression may be different | Brosché et al. (2005) |
| *Arabidopsis thaliana* and *Thellungiella halophile* under salt stress | Metabolomics (GC-MS, polar and apolar metabolites) and transcriptomic | Identification of genes and metabolites used for two species in response to salt stress | Gong et al. (2005) |
| *Phaseolus vulgaris* (plant), *Rhizobium tropici* (simbyont) under different phosphorus availability | Metabolomics (GC-MS, PEM polar metabolites), transcriptomics | Different levels of P affects the gene expression of plant<br>In P-deficient nodules aa decreased while organic and polyhydroxy acids increased | Hernández et al. (2009) |
| *Arabidopsis thaliana*, N and S stress | Metabolomics (FT-ICR-MS, polar and non-polar metabolites) and transcriptomics | General change of N and S metabolism detected in different plant organs | Hirai et al. (2004) |
| *Arabidopsis* sp under S stress | Metabolomics (FT-ICR-MS, HPLC-DAD, PEM polar metabolites) and transcriptomics | ↑ Anthocyanidin synthesis | Hirai et al. (2005) |
| *Lycopersicon esculentum* (plat) and *Tetranychus urticae* (animal) | Metabolomics (GC-MS, PEM volatile metabolites), transcriptomics | ↑ Genes of biosynthesis of mono- and diterpenes and genes of phospholipid metabolism<br>↑ Emissions on monoterpenes that increased the olfactory presence of predators | Kant et al. (2004) |
| *Arabidopsis thaliana* under S stress | Metabolomics (GC-MS, PEM polar metabolites) and transcriptomics | Elucidates the response gene-metabolite network from the transcript and metabolomic profile using mathematical algorithms | Nikiforova et al. (2004, 2005b) |
| *Lotus japonicus*, salt stress studied in different experiments with different experimental conditions | Metabolomic (GC-MS, PEM polar metabolites), transcriptomics | Large fraction of the transcriptional and metabolomic responses to salt stress was not reproducible between experiments | Sanchez et al. (2010) |
| *Solanum tuberosum* spp. *andigena* and *Solanum* spp. *tuberosum*, drought stress | Metabolomics (GC-MS, PEM polar metabolites), transcriptomics | Different gene expressions between two species ↑ proline, trehalose, GABA | Vasquez-Robinet et al. (2008) |
| *Arabidopsis thaliana*, *Pseudomonas syringqae* | Metabolomics ($^{1}$H NMR, fingerprinting, GC-MS PEM apolar metabolites), transcriptomics | ↑ aa, Glucosinolates, phenolics<br>Pathogen bacteria were able to superimpose the plant defensive strategy. The study enables to distinguish metabolic pathways that are transcriptionally activated from those that are post-transcriptionally activated | Ward et al. (2010) |

Polar fraction: amino acids and organic acids (citric, malate, etc.), mono-, di- and trisaccharides, inositol, sucrose, phenolics

Lipophilic (non-polar) fraction: fatty acids and their derivates, hydrocarbons, alkaloids, flavonol aglycones, triterpenoids, steroids

*PEM* partial ecometabolomic study, *MF* metabolic fingerprinting study, *HPLC* high pressure liquid chromatography, *MS* mass spectroscopy, *TLC* thin layer chromatography, *FT-IR* Fourier transform-infrared spectroscopy, *$^{1}$H NMR* nuclear magnetic resonance of $^{1}$H, *$^{13}$C NMR* nuclear magnetic resonance of $^{13}$C, *GC* gas chromatography, *UPLC* ultra-performance liquid chromatography

studies on the effects of C:N:P ratios on the ratios of different metabolic products such as proteins and RNA, and on growth rates and body sizes in different taxa and ecosystems (Peñuelas and Sardans 2009a). A promising way to couple stoichiometry with phenotypic metabolic expression can now be provided by ecometabolomic studies. Ecometabolomics should thus help to interpret the response of different groups of organisms in allocating resources to growth, storage and defense. It may also provide the elemental and metabolic budgets for different species along gradients from low to fast growth, which would allow a better test of the links between the C:N:P ratio, growth rate

and body-size spectrum. Similarly, ecometabolomics can be a promising tool in the frame of metabolic theory of ecology (MTE) (Brown et al. 2004). MTE states that body mass and body temperature together predict per capita rates of metabolism, respiration, growth and resource consumption, and that these rates can be scaled up to the level of populations and communities (West et al. 1999; van der Meer 2006). Ecometabolomics will allow evaluating the change in the metabolism allocation to different general functions (respiration, growth, storage) in response to different environmental situations and changes.

## More on biotic relationships

Ecometabolomic studies still need to be used to investigate other important relationships, such as plants-higher herbivores (e.g., mammals) or herbivore-predator. Moreover, most ecometabolomic studies have focused on the metabolic shifts in only one of the members of the relationships. Future ecometabolomic studies should investigate the metabolic shifts in the two species that interact simultaneously in order to have a whole vision of the phenotypic consequences of the biotic relationship. For example, some current studies aimed to study the metabolome shifts of both plants and herbivores (insect) have observed novel and useful information demonstrating the herbivore capacity to use plant metabolites to further defend itself against predators (Jansen et al. 2009). Similar reasoning can be applied to plant-pathogen, plant-symbiont or plant-vertebrate interactions, and in general to all key ecological relationships between two or more organisms.

The possibility of studying the metabolome traits and changes of more complex trophic relationships, such as three or more trophic levels at once, under different environmental circumstances, and if possible in field conditions, remains a future challenge for ecologists. For instance, the study of Harvey et al. (2003) using conventional analytical methods is promising and suggests that the study of complex trophic relationships can take advantage of modern ecometabolomic approaches. These authors, by analyzing water-soluble glucosinolates, studied the complex interactions of four trophic levels, observing that the leaf glucosinolates of *Brassica oleraceae* and *Brassica nigra* affect the development of the hyperparasitoid as mediated through the herbivore and its primary parasitoid. *B. nigra* has a more than 3.5 times higher level of glucosinolates than *B. oleracea*. Thus, there is a greater constraint in the size and survival of primary and secondary parasitoids reared from herbivores feeding on *B. nigra* than in those reared from herbivores feeding on *B. oleracea*. These results demonstrate that herbivore diet can affect the performance of interacting organisms differently across several trophic levels, and suggest that bottom-top web structure and energy fluxes can be mediated by the quality of food sources.

Some studies suggest that symbiotic fungal endophytes control insect host-parasite interaction webs (Omachi et al. 2001; Hartley and Gange 2009). Ecometabolomic studies could characterize the metabolic pathways involved in these complex relationships. Some biotic relationships are mediated by exudated metabolites; the rhizosphere is a case in point. The ecometabolomics of the rhizosphere provides another interesting perspective that also merits future study because the chemical plant and microbial exudates found there have been shown to be a key factor in the regulation of plant-microbe relationships (Micallef et al. 2009; Biedrzycki and Bais 2009) and in absorption processes (Dessureault-Rompre et al. 2007).

Other relevant future applications in the context of multiple trophic relationships involve the analysis of highly significant abiotic gradients such as water availability in terrestrial ecosystems or temperature in aquatic ecosystems. This should provide a global perspective of the phenotype responses that are the most adequate to each environmental change and of the capacity of different species to respond to environmental changes by a plastic metabolome response. In this regard, ecometabolomics may provide insight into species' stress tolerance, resistance and avoidance, giving information about species' capability to change their lifestyle and their capacity to respond to environmental changes such as severe drought, direct competition or perturbations. Ecometabolomics provides the possibility of studying different species' capacity to respond to different environmental factors at short term and to study the effects of those changes in chemical composition onto the trophic webs. Finally, this will increase our knowledge of the mechanisms underlying species composition shifts in ecosystems in response to changes in environmental conditions.

Species competition is an important ecological field where ecometabolomic studies have a promising future because, as mentioned above, only few studies have been conducted, and the results suggest interesting species mechanisms in interspecific competition. Ecometabolomics provides a useful approach to the understanding of the mechanisms underlying competitive relationships. For example, as we have discussed earlier, Peiris et al. (2008), in a ecometabolomic study of the competition among three different fungus species competing during wood decomposition, reported that 2-methyl-2,3-dihydroxypropionic acid and pyridoxine are synthesized by some fungi to inhibit the growth of their direct competitors. Thus, the competitive advantage that could be attributed to simply a greater growth capacity of one species compared to the others is in fact linked, at least partially, to a chemical growth suppression of the competitor species. However,

competition is not only an interspecific question. Intraspecific competition is also important in several ecological scenarios, and ecometabolomics is a novel tool to be used to discern which metabolome changes occur in individuals submitted to different levels of intraspecific competition.

Animal behavior is fundamental in the performance of several ecosystems; mutualism, reproductive phenology and several other behavioral phenomena are species characters that affect the performance and structure of whole ecosystems. Still, the mechanisms underlying such animal behavior are as yet not well known—which are the metabolome shifts when behavior changes and which are the implications of organism body composition? Ecometabolomics can contribute considerably in this area.

## From individuals to populations and ecosystems

Ecometabolomic applications are not only limited to the ecophysiology of organisms. Some studies upscale the use of metabolomics from individual to population and ecosystem levels. For example, Davey et al. (2008) have shown that metabolite fingerprinting and profiling is sufficiently sensitive to be able to identify the metabolic differences between populations of Arabidopsis petraea. They found two- to fourfold differences in many free amino acid concentrations between different populations. Many free carbohydrate concentrations were also different, while polyhydric alcohol concentrations were not. A principal component analysis of metabolite fingerprints revealed different metabolic phenotypes for each population. At the landscape level, Gidman et al. (2006) have shown that different metabolic fingerprints measured with rapid Fourier transform-infrared spectroscopy in tissue samples of *Galium saxatile* are correlated with a gradient of N deposition across the entire UK landscape. Ecometabolomics thus allows the investigation of complex ecological systems and provides a rapid and sensitive indicator of ecosystem health. Viant (2007) has gone a step further, using the same argument as in the origin of metabolomics: namely, that the measurement of multiple metabolites (versus one or a small group, such as occurs in classical analytical approaches) can provide a more robust assessment of the metabolic health of an organism, and hence characterizing the health of multiple species will provide a more complete assessment of ecosystem responses to environmental stressors, and even of the nature of the stressor. Ecometabolomic studies may also be used to differenciate between genetically modified and non-modified plants.

## Global change

Ecometabolomic studies of the effects of environmental gradients or of manipulation experiments in the field on various species should help to assess the impacts of the different components of global change on natural ecosystems, namely climate change, atmospheric composition changes, pollution, invasiveness and loss of biodiversity, among others. Ecometobolomic studies can help to discern species'and communities' capacity for adaptation to global changes by highlighting the metabolic pathways that are inhibited or stimulated, and by coupling with transcriptomics to determine what genes are involved in adaptation-evolution mechanisms. Moreover, the knowledge of chemical body changes that can affect the performance of trophic chains aids in understanding shifts in ecosystem structure. The effects of these global changes can only be understood by using natural environmental gradients or field manipulation experiments that allow studying the responses of species and biotic relationships, such as predation, herbivorism or parasitism, in conditions that closely resemble actual environments.

Ecometabolomic studies of organisms growing while subject to different levels of pollutants are especially important to understand organism responses to these pollutants. This is particularly true since the mechanism of action of toxins is frequently based on chemical interactions in the cells that affect metabolic expression. Moreover, ecometabolomic studies can help to discover the metabolic pathways of these chemotoxic compounds. In this vein, studies on the hyperaccumulation of trace elements in plants to discover genotypes that accumulate large amounts of certain trace elements are another example where ecometabolomic studies could be useful. Hyperaccumulator plants are an important tool in phytoremediation and soil restoration strategies, and the use of ecometabolomic studies could facilitate understanding the mechanisms of phenotypic expressions that allow plants to become hyperaccumulators.

Invasiveness is another current ecological problem of increasing global importance. When an ecosystem is invaded by one or more invasive species, some studies have reported changes in ecosystem or body composition stoichiometry (Hughes and Uowolo 2006) and in some groups of metabolites (Llusia et al. 2010; Sardans et al. 2010; Peñuelas et al. 2011). Ecometabolomic studies can help to determine what metabolic pathways and target metabolites are involved in alien success and in the resistance capacity of native species. One example of the possibilities of using ecometabolomic studies in this field is in the advance in the knowledge of the suitability of the increased competitive ability hypothesis (EICA). EICA proposes that invasive plants may still experience attack by local generalist herbivores (Müller-Schärer et al. 2004), but not by specialist herbivores. In this way, selection may favor a reduction in the expression of chemical defenses, which are effective against specialist herbivores, but metabolically demanding, and an increase in the concentrations of less costly qualitative defenses, which may be

more toxic to generalist herbivores (Joshi and Vrieling 2005; Stastny et al. 2005; Peñuelas et al. 2010). Using ecometabolomic studies of native and alien plants and of generalist and specialist herbivores conducted in the field and with an accurate use of methodologies can clarify this debate by showing the plant and herbivore metabolome changes and the differences in metabolic adaptation of herbivores to plant sources. Moreover, ecometabolomics will also allow the comparison of the metabolomes of alien plant populations, in their novel habitat, without their specialist herbivores, with the metabolomes of populations of the same species growing in their natural endemic habitat with both their generalist and specialist herbivores.

The loss of biodiversity at a global scale warrants further studies to discern its causes and its consequences. In some cases, the causes are obvious and direct: loss of habitat surface or habitat fragmentation, overexploitation of the resources or direct hunting. But in other cases, the causes are still controversial. For example, decreases in the biodiversity of pollinators in Europe and in other parts of the world are a current hot topic. As far as we know, there are no ecometabolomic studies investigating plant-pollinator metabolomes. Knowledge of changes in the metabolome in the field under climatic or pollutant stress gradients, for example, of pesticides, could be very revealing in the study of the causes and the mechanisms responsible for the decrease in pollinators in some areas of the world.

## Challenges

A serious challenge for ecometabolomic studies is to satisfy the need to disentangle the biologically relevant functions and response shifts under environmental changes, which implies determining and quantifying the maximum number of metabolites as possible. The exact number of metabolites remains a mystery, even in the case of microorganisms with simple and well-understood metabolisms. Typical non-plant eukaryotic organisms are estimated to contain from 4,000 to 20,000 metabolites (Fernie et al. 2004), and the plant kingdom produces 100,000–200,000 different metabolites (Fiehn et al. 2001), although the actual number present in any individual plant species is still unknown. Furthermore, the metabolome changes continuously, an additional challenge that is accentuated when measuring the metabolomes of several individuals from a free-living population, which will necessarily include considerable metabolic variation. There will be high levels of variation in metabolite concentrations between individuals, owing to differences in individual genetics, gender, age, organs, health status, and spatial and temporal environmental changes. Simple issues such as the time since the animal last ate or the plant last received sunlight may also be determinant. The treatment of the large temporal and individual variability found in metabolomes, which may tend to mask the sources of variation that are of interest for ecologists, can be successfully approached by trying to 'film' temporal changes in metabolite levels and their turnover rates (Peñuelas and Sardans 2009b) instead of merely taking 'snapshots' of metabolite levels, and by multiplying the number of individuals sampled. The continuous development of new advances in in vivo NMR spectroscopy and imaging, proton-transfer-reaction mass spectrometry or isotope labeling, and in the treatment of large data sets in bioinformatics will be of great assistance in this line of work (Gehlenborg et al. 2010).

Another challenge to face up to is the risk that the overwhelming '-omics-type' information reaches a field that is conceptually not properly prepared, thereby leading to the loss of an opportunity to advance ecological knowledge. Certain explanatory principles accounting for the complexity of living organisms and their populations and ecosystems, as well as of their responses to the environment, are still lacking. Ecometabolomics will thus need to focus on conceptual advancement and functional trait discovery, and not just on technological development, if it is to shed light on the fundamental system-biological mechanisms at work on scales ranging from the individual to the ecosystem. Certainly, the use of multitrophic interactions by ecometabolomics is a complex task requiring the coupling of field studies to metabolomic studies of the organisms involved in the study. This requires interdisciplinary approaches to relate the changes observed in fied measurements (growth, density, reproduction, predation, etc.) with those observed in organisms' metabolomes.

Ecometabolomic techniques have proven to have enough sensitivity for ecological studies of the metabolome response of diverse genotypes under different environmental conditions. However, the lack of past experiments in the field focused on the effects of several factors on the organism metabolome limits at present the findings of metabolome studies from an ecological perspective. On the other hand, the experimental designs of future studies should aim to also disentangle the causes of the metabolome shifts. For example, Robinson et al. (2007) observed a strong relation between the anatomical and physiological changes and the metabolic profile changes in *Pseudosuga menziesii* growing in different localities with different climate and soil traits. Although the study design did not allow a quantitative separation of the different environmental factors, it had the sensitivity to detect the environmental differences in soil type and climate.

A great drawback to dealing with all these challenges comes from the fact that ecometabolomic techniques imply the use of sophisticated and expensive equipment (GC-MS,

HPLC-MC, HNMR), which are not always available in the laboratories where ecologists work. However, the present rapid improvements in analytical methods and in the ability of computer hardware and software to interpret large data sets multiply the possibilities of rapidly identifying and quantifying simultaneously more and more compounds with great facility, even for non-specialists in this field. On the other hand, the increase in interdisciplinary research will allow a progressively wider use of these molecular techniques in ecology studies. In the future we envision increasing use of these methodologies in ecological studies. Metabolome studies coupled with transcriptomics and genomics studies, together with statistical analysis improvements and with mathematical modeling (Lindon et al. 2003), should allow a more holistic vision of the organism, population and ecosystem structure and functioning both on a space and time scale, with a better understanding of the consequences of environmental changes from individual to ecosystem level. In a step forward and unlike classical target analytical methods, ecometabolomic studies allow the characterization of the complete metabolome shift and thus help to discern the parts of the genome involved in these relationships. In this way these studies improve our knowledge of ecological consequences throughout the trophic chains, at the adaptation-selection-evolution level.

As mentioned in the analytical techniques section, the different analytical techniques have different capacities to determine different analytical groups (polar-non-polar, volatiles-non-volatiles) and different sensitivities and elucidation powers. On the other hand, the lack of extensive data bases to help in the molecular structure determination is increasingly being solved by the continuous enhancement of available commercial informatic programs and databases (Hall 2006; Allwood et al. 2008). This especially impacts the study of the plant metabolome due to the large number of secondary metabolites. Moreover, the use of several analytical methods to analyze the same organism extracts has been successfully used in some recent studies using $^{1}$HNMR and GC-MS (Srinivasan et al. 2008; Jones et al. 2009) and $^{1}$HNMR and HPLC-MS (Schroeder et al. 2006; López-Gresa et al. 2010) by thus coupling the greater sensitivity of chromatographic methods with the greater elucidation power of HNMR. In this regard the recent HPLC-DAD-MS-SPE-NMR provides high sensitivity and elucidation power at once (Schlotterbeck and Ceccarelli 2009).

If ecological metabolomics succeeds in overcoming these challenges and uses them as opportunities for advancing knowledge, we can expect to see stimulating new developments and applications in the near future in many areas of ecological sciences, including issues of stress responses, life history variation, population structure, trophic interaction, nutrient cycling and the ecological niche. For example, the temporal and spatial characterization of the responses of individuals, populations and ecosystems to perturbations such as global change and the disentangling of evolutionary aspects of plant and animal communities both offer ecological metabolomics an immediate opportunity as a new and exciting application. In turn, ecology can provide a unique insight and a significant contribution to the study of functional metabolomics by helping to understand the ecological basis for interactions among metabolites.

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