Commentary

Climate change and ecosystem function – full-scale manipulations of CO_2 and temperature

On the bookshelf above my head I can see the book *Biogeochemistry of a forested ecosystem* (Likens *et al.*, 1977). It is in a pretty bad shape, worn out after intensive use for many years. The book describes the ecosystem studies in Hubbard Brook, NH, USA (http://www.hubbardbrook.org/). To me this was the first practical example and pioneer of a true multidisciplinary 'whole-ecosystem' approach to study and understand ecosystem processes and functioning. Investigating the effects of external impact factors on the ecosystem by conducting a full-scale manipulation experiment and subsequently follow the consequences at various scales – it has been a strong inspiration to me ever since. In this issue of *New Phytologist*, a range of research studies addressing ecosystem-level studies are showcased, and critical reviews cover FACE (Free Air CO_2 Enrichment) experiments to study elevated CO_2 and effects of elevated CO_2 and climatic change on overall ecosystem functioning (Norby & Luo, pp. 281–293), phenology (Badeck *et al.* pp. 295–309) and below-ground processes (Pendall *et al.* pp. 311–322).

> *'The few examples of combinations of CO_2 and warming point in all directions and results are not predictable based on the individual effects'*

The ecosystem approach

Hubbard Brook is still going strong, but it is no longer alone. Many ecosystem studies have been conducted since then. In particular, this ecosystem approach took a major step forward during the 'acid rain' era, where many ecosystem studies including acid and nutrient manipulations were conducted in Europe and the USA. These experiments included large-scale manipulation projects such as the Norwegian RAIN project (Wright *et al.*, 1993), the European EXMAN and NITREX projects (Wright & Rasmussen, 1998) the Gårdsjøn roof project in Sweden (Hultberg & Skeffington, 1998), and the whole watershed manipulations in the US at the Bear Brook Watershed in Maine (Norton & Fernandez, 1999) and the Fernow Experimental Forest in West Virginia (Gilliam *et al.*, 1996).

Over the years, the whole-ecosystem approach has proved particularly valuable and unique as a tool for investigating complex cause-effect relationships in ecosystem internal processes in natural environments (Beier & Rasmussen, 1994). Despite the complexity and spatial/temporal variability of ecosystem processes, field-scale manipulation experiments coupled with process, species, community and ecosystem level studies have become an important tool for generating knowledge about ecosystem processes and responses. Furthermore, ecosystem manipulation experiments are unique for validating and testing dynamic model predictions. If a model is applied to the untreated control situation at the site and subsequently used to predict the effects of the applied manipulations, the model output can be evaluated by comparison with the measured responses in the treatment (Ferrier, 1998; Beier *et al.*, 2003). This provides much stronger confidence in the model's ability to predict effects of environmental changes compared to a 'standard' situation where the dynamic behaviour of the model can only be evaluated from temporal fluctuations.

Ecosystem experiments and climate change

Climate change is another large-scale threat to the environment, and one that has gained increasing scientific and political attention over the past two decades. Climate change is particularly complex compared with other previous regional and global environmental changes because it involves simultaneous increases in atmospheric CO_2 and temperature (IPCC, 2001), coupled with altered precipitation (Weltzin & Tissue, 2003). Together these are among the most important factors directly involved in regulating biological and chemical processes and can cause a whole cascade of effects from the individual organism all the way up to the ecosystem scale. In order to understand and predict the potential effect of this complexity of changes a large number of research projects have been carried out involving studies of climate driven changes at all ecosystem scales and involving all levels from single process studies in laboratories and controlled environments to full-scale ecosystem studies in natural environments. Ultimately, this should provide the necessary background needed to predict long-term ecosystem responses to climate change.

Because of the complexity and costs for large-scale ecosystem studies, the majority of these have focused on single factors. For example, a large number of warming studies were carried out by various techniques in the 1990s (synthesised by Rustad *et al.*, 2001) and a number of large scale CO_2 enrichment studies have been carried out by the FACE technique, synthesised in this issue by Nowak *et al.* These studies have together provided an important input to our understanding of how biological processes will respond to predicted climatic changes (Shaver *et al.*, 2000). The bulk of these research projects have also underscored the fact that ecosystem responses to changes in climatic and environmental factors are highly variable and complex. Furthermore, it is clear that these single or few-factor experiments may not provide a comprehensive understanding of how ecosystems behave when all factors change simultaneously.

A multifactor world, a multifactor problem

Together, the papers in this issue clearly illustrate the value of a mechanistic understanding of how changes in the various climatic factors will affect specific ecosystem processes. However, they also clearly illustrate the difficulty of obtaining a true functional understanding of ecosystem responses based on single factor and single process studies alone. The effects of CO_2 alone and to a lesser extent warming alone each show some general and consistent patterns, but the few examples of combinations point in all directions and results are not predictable based on the individual effects. The complexity and unpredictability becomes even worse when we realise that important effects may be driven by changes in off-season processes, seasonality and extreme events – as illustrated in the paper by Loik *et al.* (pp. 331–341) showing how warming affects the freezing tolerance for certain species.

Obviously, the solution to this problem may include two important elements: multifactor experiments and modelling. To date, very few ecosystem experiments have been conducted involving combinations of all or several climate change parameters. The CLIMEX project conducted in Norway in 1995–1999 (Wright, 1998) was the first full-scale ecosystem experiment in which a complete boreal forest catchment was exposed to simultaneously elevated CO_2 and temperature, and showed changes in N mineralisation processes and N leaching (Wright *et al.*, 1998).

Recently, results from the climate change experiment at Jasper Ridge, CA, USA have clearly demonstrated how complex and unpredictable ecosystem responses may be in a multifactor world. The annual grassland was exposed to combinations of elevated CO_2 and temperature, and changes in precipitation and nitrogen deposition. The results show that while all treatments involving increased temperature, precipitation or N deposition (alone or in combination) as well as CO_2 alone tended to promote the above ground biomass production and net primary productivity (NPP) in the ecosystem, all combinations of elevated CO_2 with the other treatments dampened the increase (Shaw *et al.*, 2002).

Climate change and ecosystem research – our future challenge

The research in this issue suggests a general consensus that a combination of ecosystem-scale experiments and modelling is needed. At the same time opinions are mixed regarding the relative importance of these elements. Dynamic ecosystem models will be useful for integrating our understanding from the individual process studies and thereby provide common tools for forecasting future short- and long-term changes in ecosystem functioning as claimed by Norby & Luo. On the other hand, this is still a major challenge and will only be useful if the general understanding of how the individual factors interact is good enough. For this, multifactor experiments at the ecosystem scale are crucial both to generate the knowledge needed to build the models and to test and validate the results. A way to optimise the resource use and improve the generality is to combine experiments and gradients by conducting the same ecosystem experiments at different or comparable ecosystems along climatic gradients. This will contrast or combine the short-term effects obtained through experiments at any particular site with knowledge of long-term differences and stability of the ecosystem processes along the climatic or environmental gradient. This will further provide insight into the route the ecosystem and ecosystem processes will take moving from the present to the future state. This strategy was used in the European CLIMOOR and VULCAN projects (Beier *et al.*, 2004) and shows how variable effects of droughts and warming are among sites under different climatic conditions (Emmett *et al.*, 2004; Peñuelas *et al.*, 2004).

The problem is that multifactor experiments at the ecosystem scale are generally extremely resource demanding, particularly if they are to be continued for sufficient time to provide information about the longer-term responses. Furthermore, as Norby & Luo state: they will always be case studies. On the other hand, do we have an alternative? I doubt it. I do not believe that any model alone or any single factor experiment will provide the answers we need to predict long-term ecosystem responses to future changes in climatic and environmental factors.

There is a crucial need to conduct long-term ecosystem-scale multifactor experiments including changes in the main drivers we know are going to change in the future and to do this across ecosystems and climatic zones. This is a multidisciplinary challenge and each study should involve scientists covering all the ecosystem scales from individual processes to ecosystem structure and function.

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Letters

Enzymatic sources of nitric oxide in plant cells – beyond one protein–one function

In the mid 1970s it was reported that plants can emit nitric oxide (NO·) (Klepper, 1979) and nowadays the generation of NO· in plant cells is well established (Leshem, 2000; Lamattina *et al.*, 2003; Neill *et al.*, 2003). In plants, like in animal systems, the gaseous free radical NO· has been shown to play a role as a signal molecule in diverse important physiological processes (Delledonne *et al.*, 1998; Wendehenne *et al.*, 2001; Lamattina *et al.*, 2003; Neill *et al.*, 2003). However, the enzymatic source and site of NO· synthesis in plant cells has been the subject of much debate and controversy.

In the past decade, many plant biologists searched intensively for an enzyme similar to any of the isoforms of nitric oxide synthase (NOS) identified in mammalian systems (Alderton *et al.*, 2001), in the hope of finding the gene responsible for the 'plant NOS'. A significant number of reports showed the presence of NOS-like activity in several plant tissues which had some similiarities with mammalian NOS, such as sensitivity to well characterized NOS inhibitors and cross-reactivity with several antibodies against mammalian NOS proteins. But following the publication of the *Arabidopsis* genome, not a single gene or protein with sequence similarity to the animal NOSs could be identified (The Arabidopsis genome initiative, 2000). Therefore, alternative enzymatic sources of NO· had to be considered. The previous demonstration of the NO· production by the enzyme nitrate reductase (Dean & Harper, 1988; Yamasaki *et al.*, 1999) was one possibility (García-Mata & Lamattina, 2003). However, recently an unexpected alternative enzymatic source of NO· was described. A variant of the P protein of the mitochondrial glycine descarboxylase complex (GDC) was demonstrated to be a pathogen-inducible plant NOS (Chandok *et al.*, 2003; Wendehenne *et al.*, 2003). Additionally, another recent report has shown the presence of an NOS gene (*AtNOS1*) in *Arabidopsis*. AtNOS1 turned out to be a protein very similar to a group of bacterial proteins with putative GTP-binding or GTPase domains (Guo *et al.*, 2003). Surprisingly, both the variant P protein and the purified AtNOS1 protein did not have sequence similarities to any mammalian NOS.

However, apart from these cases, there are still other potential enzymatic sources of NO· generation in plants that must be considered (Table 1). The production of NO· by horseradish peroxidase from hydroxyurea and H_2O_2 (Huang *et al.*, 2002) is an example of how plant cells can have alternative sources of NO· making use of the widespread and physiologically important enzymes peroxidases (Huang *et al.*, 2002). Other enzymatic sources that must be taken into account are xanthine oxidoreductase and cytochrome P450 which are present in plants and have been shown to generate NO· in animal systems (Boucher *et al.*, 1992a; Millar *et al.*, 1998; Harrison, 2002; Mansuy & Boucher, 2002). Moreover, it has been proposed that hemeproteins are good candidates for the enzymatic generation of NO· from *N*-hydroxyarginine (NOHA) (Boucher *et al.*, 1992b). Additionally, a plasma membrane-bound enzyme was shown to catalyze the formation of NO· from nitrite in tobacco

Table 1 Some established and potential enzymatic sources of NO· in plant cells

Source	Reference
Crude extracts and cell organelles (NOS-like activity)	Sen & Cheema (1995); Cueto *et al*. (1996); Ninnemann & Maier (1996); Delledonne *et al*. (1998); Durner *et al*. (1998); Ribeiro *et al*. (1999); Barroso *et al*. (1999); Modolo *et al*. (2002)
Variant P protein of the GDC ('plant iNOS')	Chandok *et al*. (2003)
Arabidopsis protein (AtNOS1)	Guo *et al*. (2003)
Nitrate reductase	Dean and Harper (1988); Yamasaki *et al*. (1999)
Xanthine oxidoreductase	Millar *et al*. (1998); Harrison (2002)
Horseradish Peroxidase	Boucher *et al*. (1992b); Huang *et al*. (2002)
Cytochrome P450	Boucher *et al*. (1992a); Mansuy & Boucher (2002)
Hemeproteins	Boucher *et al*. (1992b)
Unknown plasma membrane-bound enzyme	Stöhr *et al*. (2001)

roots (Stöhr *et al.*, 2001). Taken together, this suggests that in plants the enzymatic NO· production, either constitutive or induced by different biotic/abiotic stresses, may be a much more common event than was initially thought.

These examples show that the dated concept of one protein–one function is too simplistic as far as NO· generation is concerned. To improve our understanding of the physiological function of NO· in the different plant cell compartments, we must realize that plant cells may not possess a unique enzymatic source of this versatile molecule but multiple generating systems. It appears that in plants there is a battery of multifunctional enzymes, able to produce NO· catalytically, which are structurally unrelated to mammalian NOS. The burst of publications on 'plant NOS activity' over recent years, and the special interest in demonstrating that the origin of NO was due to a unique constitutive or inducible mammalian-type NOS, brings into the memory the discovery of the generation in biological systems of another important free radical, the superoxide anion ($O_2^{\cdot -}$) (McCord & Fridovich, 1968). It was then thought that $O_2^{\cdot -}$ radicals were only produced by the mammalian oxidative enzyme xanthine oxidase. Today it is known that there are many proteins and enzymes producing these radicals in many compartments of animal and plant cells, as a consequence of normal aerobic metabolism, and it is well established that the generation of $O_2^{\cdot -}$ radicals may be induced by different pathological or stress conditions (Bolwell, 1999; Dat *et al.*, 2000; Halliwell & Gutteridge, 2000; del Río *et al.*, 2003). In a similar way, concerning the enzymatic production of NO· in plants, perhaps we are just starting to see the tip of the iceberg.

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Key words: nitric oxide, NO·, signalling, nitric oxide synthase, NOS.

Meetings

Austral challenges to northern hemisphere orthodoxy

IV Southern Connections Conference: Towards a southern perspective, Cape Town, South Africa, January 2004

Southern hemisphere ecologists and biogeographers have traditionally looked northward for stimulus and ideas. Southern Connections (http://www.southernconnection.org.au) arose from the recent realization that there was also much to be gained by looking east and west. This organization has held meetings every 3–4 yr since 1994, providing a unique interdisciplinary forum for whole-organism biologists working in the southern hemisphere. One speaker at an earlier meeting commented on how refreshing it was *not* to begin by virtually apologising for his subject (terrestrial mammal pollination in Proteaceae) as he would in a northern hemisphere forum, where such mechanisms might be viewed as curiosities or, at worst, freak shows. At Southern Connec-tions he could take it for granted that many would be conversant with his subject, which could then be discussed in more depth.

The mood generates a healthy iconoclasm about 'unifying schemes' served up mainly from Europe and North America. Widely read texts were derided for presenting 'laughable' generalizations, many of which have to be unlearned by southern hemisphere ecologists and biogeographers. Thus, a mission of Southern Connections is to ensure that scientific generalizations and theory genuinely address both hemispheres.

Notwithstanding the common Gondwanan past of their respective lands, and the resulting biotic affinities, there are other issues that unite the scientists involved. Many of these countries are facing similar issues arising from rapid changes in land use, massive introduction of alien species and declining native biodiversity. Although a wide range of topics in ecology, biogeography and systematics were presented at the IV Southern Connections Conference (http://web.uct.ac.za/conferences/sc2004), below we concentrate on some of the major issues in plant ecology addressed at the meeting.

> *'Are southern hemisphere ecosystems susceptible to invasion by Eurasian taxa because northern hemisphere plants are simply "better"?'*

Invasive plants – correlates and consequences

Huge numbers of exotic plant species have been introduced to southern hemisphere countries. Duane Peltzer (Landcare Research, Lincoln, New Zealand) showed us an extreme case in New Zealand, where naturalized exotic vascular plants now outnumber natives. Although by no means all naturalized plants can be classed as invasive, it is therefore no surprise that the ecology of invasions is a big research topic in the southern hemisphere.

Are southern hemisphere ecosystems susceptible to invasion by Eurasian taxa because northern hemisphere plants are simply 'better'? This idea, referred to by Ian Radford (Otago University, New Zealand) as the 'biological cringe', was not supported by an analysis of 233 invasive plant species in South Africa, carried out by Lesley Henderson (Agricultural Research Council, Pretoria, South Africa). She found that exotics originating from the northern and southern temperate zones had identical average rates of expansion in that country.

Peter Williams (Landcare Research, Nelson, New Zealand) highlighted the importance of propagule pressure and suitability of the abiotic environment in determining naturalization success of plants in Australia and New Zealand. The current prevalence of Eurasian species in New Zealand's register of exotic plants therefore reflects the persistent efforts of 19th century colonists to create a "Better Britain" in the Antipodes', as well as an influx of Eurasian stowaways. Suitability of environment was also a good predictor of which species invade successfully in different parts of South Africa (Mathieu Rouget, Kirstenbosch Botanic Gardens, South Africa). However, the case of *Eucalyptus* shows that massive propagule pressure and suitable abiotic environments won't turn all exotics into invasives. Dave Richardson (University of Cape Town, South Africa) showed that despite widespread plantings around the world, most *Eucalyptus* species scarcely naturalise and very few are aggressive invaders.

In many cases, invasive plants are most prominent in early successional communities, eventually giving way to late-successional natives. However, in those cases where their functional traits differ from those of competing native pioneers, their influence on ecosystem properties such as soil nutrient availability could leave a persistent legacy. Duane Peltzer and Peter Bellingham (Landcare Research, Lincoln, New Zealand) described successions in New Zealand, where exotic pioneer shrubs such as *Buddleja davidii* and *Ulex europaeus* produce more nutrient-rich leaf litter than their nearest native equivalents such as *Coriaria arborea* and *Kunzea ericoides*. Elizabeth Lindsay and Tanya Mason (University of Wollongong, Australia) presented similar results from contrasting the exotic shrub *Chrysanthemoides molinifera* and native species such as *Banksia integrifolia*. Successional trajectories are in some cases quite different under exotic and native shrub canopies, and as many of the species regenerating beneath them live for several centuries, the impact on vegetation composition at landscape level could be appreciable.

Mutualism collapse

The proportion of woody plants dependent on vertebrates for pollination and seed dispersal is generally higher in southern temperate forests than in their northern counterparts (Armesto *et al.*, 1996; Willson *et al.*, 1996). This dependence is even higher on some tropical Pacific islands such as Tonga and New Caledonia (McConkey & Drake, 2002). Community structure and ecosystem function are therefore likely to be particularly sensitive to extinctions in these systems.

Alastair Robertson (Massey University, New Zealand) reviewed collapsing plant–bird mutualisms on Pacific islands, where human settlement has resulted in dozens of bird extinctions, including many frugivores (Steadman, 1995). New Zealand has lost 13 frugivorous birds since human arrival during the last millennium (Worthy & Holdaway, 2002). Although this is not known to have caused the extinction of any plant species to date, a dozen large-seeded woody plants now depend on a single vector, the pigeon *Hemiphaga novaezelandiae* (Clout & Hay, 1989), whose populations have dwindled in the face of depredation by introduced mammals as well as humans. Similarly, the extinction of four pigeon species (*Ducula* spp.) in Tonga has left plants with diaspores larger than *c.* 28 mm in diameter without any known seed disperser (McConkey & Drake, 2002). Pollination mutualisms are also vulnerable throughout the Pacific region, as a result of recent declines in abundance of important vertebrate pollinators such as honeyeaters and bats.

Robertson quantified the effects of local extinction or decline of mutualists on seed dispersal and set of several plant species in New Zealand. Reproductive success was consistently poorer in plant populations on mainland New Zealand than on small islands where conservation programmes have retained dense populations of pollinators and seed dispersers. Similarly, Anton Pauw (University of Cape Town, South Africa) made use of spatial and temporal comparisons to show how orchid pollination in South Africa has been impaired by declines in abundance of oil-collecting bees.

Although most previous studies have focused on the vulnerability of plants to loss of their pollinators or dispersers, Cecilia Smith-Ramirez (Universidad Católica de Chile, Santiago) reminded us not to overlook the situation of specialist pollinators. Her analysis of the pollinator assemblage of the Chilean rainforest tree *Eucryphia cordifolia* (currently a common species) revealed 15 species of *Diptera* which have not been reported visiting flowers of any other plant.

> *'25% of the Earth's vegetation is fire-maintained'*

Fire – controlling global vegetation patterns

Studies of South African and Australian ecosystems have produced novel perspectives on the role of fire as a driver of plant evolution, and as a control on global vegetation patterns. It has long been recognized that adaptations facilitating regeneration after fire are common in some floras. A more recent suggestion is that flammability can be advantageous to a given genotype if it promotes the spread of fire to neighbours lacking sprouting or seeding responses that permit rapid recapture of the site (Bond & Midgley, 1995).

The Sheffield Dynamic Global Vegetation Model (SDGVM: Woodward *et al.*, 1995) generates the provocative prediction that 25% of the Earth's vegetation is fire-maintained. The SDGVM uses physiological criteria to predict spatial and temporal variation in broad vegetation types on the basis of climate, soil and atmospheric CO_2 concentration. William Bond (University of Capetown, South Africa) showed that the model grossly overpredicts global forest cover, unless a fire module is incorporated in simulations. Without fire, forest cover is predicted for Mediterranean-type climates, implying that widespread heathlands and shrublands in those regions are fire-maintained. When fire is added to the model, the predicted global vegetation map coincides closely with current patterns, although deciduous forest is still incorrectly predicted for maritime temperate climates in Chile, New Zealand and the Pacific North-west.

Long-term fire exclusion experiments in South African savannas support model predictions (Bond *et al.*, 2003). Sites receiving > 650 mm rainfall show succession to forest when fire is excluded, whereas arid savanna sites (predicted to remain as savanna), although showing some increase in tree biomass, do not develop closed forest. An analysis of Tasmanian landscapes by Ross Bradstock (NSW National Parks & Wildlife Service, Hurstville, Australia) developed the interesting related point that low site fertility can promote fire by prolonging dominance of flammable early successional vegetation.

When and why did fire become so important? Bond *et al.* (2003) argue that the spread of fire as a major ecological force can be linked to the advent of a new fuel type: the C_4 grasses which proliferated under the falling CO_2 levels of the late Miocene. The combination of rapid growth and production of slow-decaying litter by many C_4 grasses results in rapid buildup of inflammable material, enabling these plants to carry fire into mesic habitats (D'Antonio & Vitousek, 1992). Ensuing changes in disturbance regimes may have triggered the evolution of fire-adapted traits in other taxa.

Has fire split southern biotas? David Bowman (Charles Darwin University, Australia) argued that the ecological and evolutionary impact of fire in Australian landscapes predates human arrival by millions of years (*contra* Flannery, 1994), as is also clearly so in South Africa. Peter Clarke (University of New England, Armidale, Australia) showed that fire stimulated recruitment of > 80% of taxa even on relatively mesic sites in New South Wales. Although fire seems to have played a lesser role in the evolution of South American vegetation, some plants show probable fire adaptations, such as lignotubers in central Chilean matorral (Montenegro *et al.*, 1983). We know little about prehuman fire regimes in temperate South America, but Mauro González (Universidad Austral de Chile, Valdivia), Donaldo Bran (INTA EEA, Bariloche, Argentina) and Tom Veblen (University of Colorado, Boulder, CO, USA) showed how present vegetation patterns in some parts of the region have been shaped by fire. González also pointed out that fuel loads associated with synchronous flowering and death of *Chusquea* bamboos promote rapid spread of forest fires, whether of human or natural origin.

Whether as a result of climate or isolation from evolutionary innovations, fire seems to have made fewer inroads in New Zealand than in any of the major southern landmasses. Geoff Rogers (Department of Conservation, Dunedin, New Zealand) estimated prehuman fire return intervals of > 1000 yr in the eastern South Island, attesting to the low flammability of vegetation even in this relatively dry district of the country. Although human arrival greatly increased fire frequency, most native plants lack the ability to recapture sites rapidly through sprouts or seed banks.

The future

While 'Towards a southern perspective' may be a reasonable short-term goal of Southern Connections, the real issue is that due consideration of both hemispheres means a better understanding of ecological processes and the evolution of the biosphere as a whole. Perhaps the clearest case is the work by South African and Australian ecologists on fire, which, although spurred mainly by studies of their own particularly fire-prone systems, may force a rethink on the part of plant ecologists and biogeographers the world over.

Another worthwhile aim must be to improve the representation of some of the smaller southern hemisphere countries at future conferences. Perspectives from New Caledonia and Madagascar were sorely missed, and efforts must be made to persuade delegates from these remarkable Gondwanic fragments to attend the next meeting in 2007 in Adelaide.

Southern Connections is a 'must attend' forum for southern hemisphere whole-organism biologists that northern hemisphere scientists might also do well to attend, to help a flow of ideas across the divide. Perhaps there is also a need for 'Northern Connections' ensuring east–west interchange and inclusion of east Asian and Russian input to ecological and biogeographic theory.

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